Nutritional Bioavailability of Zinc

Edited by

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Nutritional Bioavailability of Zinc

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

ALTHOUGH ZINC WAS KNOWN as a required mineral nutrient for the diets of animals, zinc deficiency in humans' diets was not recognized until the early 1960s. Individuals consuming an amount of dietary zinc exceeding the usual designated requirement still may show signs of nutritional zinc deficiency. Thus, the adequacy of zinc in humans' diets must be evaluated based on the bioavailability of dietary zinc.

This book is based on the symposium that was designed to assess the current perspective and future direction of research on the nutritional bioavailability of zinc. Inhibitors suspected of interfering with the absorption of zinc are some factors that influence bioavailability. Phytates, dietary fibers, proteins, nonenzymatic browning products, and certain micronutrients are among these substances. These inhibitors are covered in various chapters.

The sites of zinc absorption in the mammalian gastrointestinal tract are largely unknown. However, zinc absorption appears to be facilitated by a low molecular weight, zinc-binding ligand. Citric acid and picolinic acid are two such substances with supporting data for their ligand role. Intercellular events involve a zinc flux from mucosa-to-serosa and return. Thionein contributes to the regulation of the entry of dietary zinc from the mucosal cell into the body. These and other biochemical and metabolic aspects of zinc are explained.

Methods for studying zinc bioavailability in humans include metabolic balance studies, radioisotopic techniques, stable isotope techniques, circulating zinc response, and perfusion techniques. These methods of bioavailability, along with other zinc-related studies, also are covered in detail.

I wish to acknowledge the contributions of the authors and reviewers who made this volume possible. My thanks and gratitude are extended

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Experimental Zinc Deficiency in Humans

An Overview of Original Studies

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During the past two decades, essentiality of zinc for man has been established. Deficiency of zinc in man due to nutritional factors and several diseased states, has been recognized. A marginal deficiency of zinc appears to be prevalent in many segments of population in developed countries and more severe deficiencies are widespread in many parts of the world. In our experimental human model, a marginal deficiency of zinc was induced by dietary means. Loss of body weight (less than 10% in six months on zinc restricted diet), testicular hypofunction, hyperammonemia and a decrease in plasma, urinary and neutrophil zinc concentration were observed. Changes in zinc dependent enzymes such as deoxythymidine kinase in newly synthesized connective tissue and plasma alkaline phosphatase were also observed as a result of zinc restriction and repletion in our model.

Although the role of zinc in human subjects has been now defined and its deficiency recognized in several clinical conditions, these examples are not representative of a pure zinc deficient state in man. It was, therefore, considered desirable to develop a human model which would allow a study of the effects of a mild zinc deficient state in man. Recently such a model has been established successfully in human volunteers with the use of a semi-purified diet based on texturized soy protein.

Long-standing nutritional zinc deficiency has been reported to cause primary hypogonadism in human subjects (1, 2). Deficiency of zinc occurring in association with certain diseases has also been reported to affect adversely testicular function (3). In experimental animals, zinc-restricted diet is known to produce primary hypogonadism (4).

In both animals and human subjects, testicular hypofunction

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due to zinc deficiency was characterized by decreased function of the Leydig cells and oligospermia (3). A decrease in serum androgens, an increase in serum gonadotropins, and arrest of spermatogenesis were observed in testicular biopsies obtained from some patients who were zinc-deficient. Also, zinc supplementation has been used to increase plasma testosterone level and sperm count in infertile men (5).

In previous reports, a deficiency of zinc was believed to cause male hypogonadism, but because of the complicated nature of the clinical problems in all such cases, other factors responsible for hypogonadism, nutritional or otherwise, could not be ruled out. However, in all such cases reported so far, moderately severe deficiency of zinc was present for several years. In this study we have shown that even a mild deficiency of zinc can adversely influence testicular function in adult subjects, which was reversible with zinc supplementation.

In this paper, I will summarize our experience with this model.

Methods and Results

Four male volunteers participated in the first experiment. The first pair of two subjects (Patient 1, Patient 2) were 57 and 55-year-old white men, respectively. Patient 1 had degenerative osteoarthritis and allergic rhinitis. Results of physical examination, routine blood tests, and zinc concentration of plasma erythrocytes and hair were within normal limits before the study. Patient 2 was diagnosed as having mild diabetes mellitus and mild hypertension. At the time the patients entered the study, there were no abnormal physical findings, and zinc status was within normal limits.

The second pair of subjects were Patient 3, a 56-year-old black man who was being followed up for essential hypertension and gouty arthritis, and Patient 4, a 65-year-old white man who had chronic sinusitis and mild diabetes insipidus. At the time of the study Patient 3 and 4 were asymptomatic, with no abnormal physical or laboratory findings. Zinc status as assessed by zinc concentration in plasma, erythrocytes, and hair was within normal limits in both subjects.

After a thorough physical examination, routine laboratory tests were done, including routine hematologic tests, serum electrolytes, blood urea nitrogen, serum creatinine, and fasting blood sugar. Serum levels of calcium, inorganic phosphate, total cholesterol, triglyceride, total protein, uric acid, total bilirubin, serum glutamic-oxalacetic transaminase, vitamin A, and carotene were also measured. These routine blood tests were repeated once a month. Routine chest roentgenograms and urinalysis were carried out in the beginning and periodically as needed.

The subjects were kept on the metabolic ward, and two physicians followed them up regularly. Psychologic testing was done

by a clinical psychologist thrice, initially, once at the end of the restricted zinc intake period and again after zinc supplementation.

A semi-purified diet based on texturized soy protein (purchased from General Mills Company, Minneapolis, Minnesota (Bontrae Products) and Worthington Foods Company, Division of Miles Laboratory, Elkhart, Indiana) was developed for this study. Soy protein isolate was used as soy flour in the baked goods (purchased from General Biochemicals, Teklad Mills, Chagrin Falls, Ohio). The texturized soy meals used were hamburger granules, chicken slices, turkey slices, and chicken chunks. The texturized soy protein and soy protein isolate were washed twice with ethylenediaminetetraacetate, then rinsed three times with deionized water, boiled for 30 min. and kept frozen until ready to be used. Most recipes used in this study were adapted from Soy Protein Recipe Ideas, published by Institution Volume Feeding Management Magazine, Chicago, Illinois.

The foods were cooked in large quantities and stored in a freezer for 1 to 3 months. As needed, the food items were defrosted in the refrigerator, heated, weighed, and then served to the volunteers. Every 4 weeks for 7 days, breakfast, lunch, dinner, and snacks served each day were homogenized in a blender. Aliquots of the composite homogenized meals were weighed, frozen, lyophilized, and then analyzed for fat, nitrogen, and zinc content of the meals. For the first two subjects (Patient 1 and 2), the diet supplied 1865 kcal, 53 g of protein, and all essential vitamins and minerals except zinc, according to recommended dietary allowances. The second set of patients (Patients 3 and 4) received a vitamin tablet (Poly-Vi-Sol, Mead Johnson Laboratories, Evansville, Indiana) and a mineral mixture. The second pair of subjects also received protein supplement (Stuart Amino Acids Powder, Stuart Pharmacy, Wilmington, Delaware). The daily intake of calories was 2352 kcal and protein, 58 g.

The first pair of subjects (Patients 1 and 2) received hospital diet for 2 weeks; then they received the experimental diet with 10 mg of supplemental zinc (as zinc acetate) daily orally for 6 weeks. After this, they were given only experimental diet (daily zinc intake of 2.7 mg) for 24 weeks. At the end of this phase, while continuing the experimental diet, the two subjects received 30 mg of zinc supplement (as zinc acetate) daily orally for 12 weeks. Finally, these subjects were maintained on hospital diet with total daily intake of 10 mg zinc plus 30 mg of oral zinc supplement (as zinc acetate) for 8 weeks. The hospital diet provided the same amount of calories and protein as the experimental diet. Thus, these two subjects were observed for a total period of 52 weeks.

The second two subjects (Patients 3 and 4) received hospital diet (10 mg of zinc intake daily) for 3 weeks, followed by the experimental diet with 30 mg of oral zinc supplement (as zinc acetate) for 5 weeks. After this, they were given only experi-

mental diet (3.5 mg of zinc intake daily) for 50 weeks. The repletion phase was begun with the administration of 30 mg of zinc (as zinc acetate) orally while the same experimental diet was maintained and continued for a total period of 8 weeks, at the end of which the hospital diet replaced the experimental diet. The calorie and protein intake from the hospital and the experimental diets were the same. Oral zinc supplement (30 mg as zinc acetate) was continued along with the hospital diet for a period of 8 weeks. Altogether these two subjects were observed for 64 weeks.

Deionized water was given for drinking purposes throughout the study period. Care was taken to ensure that the diet provided the same number of calories and protein quantity throughout the study period, including the period in which the subjects received the hospital diet. Whenever appropriate, extra trays of the hospital diet (composite of 7 days) were homogenized and analyzed for fat, protein, and zinc content.

The patients were weighed three times a week, and skinfold measurements were taken periodically of the abdomen, triceps, subscapular areas, using skinfold calipers according to the procedure outlined by the manufacturer (Cambridge Scientific Industries, Inc., Cambridge, Maryland).

Urine and fecal samples were collected for 7 consecutive days every 4 weeks. Fecal samples were analyzed for nitrogen, fat, and zinc content. Fecal samples were weighed, lyophilized, digested with nitric acid, diluted to volume with deionized water, and analyzed by the atomic absorption spectrophotometer, model 303 or 306 (Perkin Elmer, Norwalk, Connecticut). Food samples were weighed, wet digested with nitric acid, and diluted to volume with deionized water, then analyzed for zinc level (6). Nitrogen levels of dried samples (food or feces) were determined by Kjeldahl procedure (7). The fat content of dried samples (food or feces) was ascertained by ether extraction of the lipids (8).

Blood samples were drawn every other week, using plastic syringes and tubes. The whole blood was centrifuged and plasma pipetted into plastic tubes, then precipitated with 10% trichloroacetic acid, diluted 1:4 with deionized water, and analyzed for zinc by the atomic absorption spectrophotometer, model 303 or 306. The erythrocytes were washed three times with normal saline. After the last centrifugation 1 ml of packed erythrocytes were pipetted into plastic tubes, and 2 ml deionized water was added. Hemoglobin level was measured using cyanide technique on Beckman DK-2 spectrophotometer (Fullerton, California). Erythrocytes were digested with nitric acid and diluted to volume with deionized water. Zinc was then assayed by atomic absorption spectrophotometer and values expressed in termes of micrograms of zinc per gram of hemoglobin. The leukocytes were separated by a technique reported by Rothstein, Bishop, and Ashenbrucker (9), and zinc content was measured by atomic absorption spectrophotometer.

For plasma ammonia levels, whole blood was centrifuged and plasma pipetted into ammonia-free tubes. The plasma was then frozen immediately and kept frozen until ammonia was measured by an autoanalyzer. Urea nitrogen in the plasma and creatinine excretion in the urine were also measured by the autoanalyzer. Lactic dehydrogenase activity in the plasma was measured by a method published by Bergmeyer, Bernt, and Hess (10). Ribonuclease activity was ascertained by a modified Sekine method (11, 12). The activity of alkaline phosphatase in the plasma was measured by a colorimetric procedure.

In each of the first two subjects (Patients 1 and 2), after local anesthesia, one polyvinyl sponge measuring approximately 4 cm x 3 cm x 3 mm was implanted subcutaneously on the lateral aspect of the chest. This was done in order to obtain newly synthesizing collagen connective tissue for assay of deoxythymidine kinase activity. Twenty-one days after implantation the sponges were isolated by blunt dissection , and the capsules surrounding the sponges were collected for study. Total protein, total collagen, ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) ratio, and the activity of deoxythymidine kinase were assayed by techniques reported previously (13). Sponge implantation was done twice, once at the end of the zinc restriction period and again after zinc supplementation for 12 weeks, while the subjects received the same experimental diet otherwise.

Clinical and psychologic evaluations during the study period remained essentially unchanged. The first pair of subjects (Patients 1 and 2), on 2.7 mg daily zinc intake, complained of mild roughening of skin and lethargy; but these were not observed in the second pair (Patients 3 and 4), on 3.5 mg of daily zinc intake.

Results of routine laboratory tests remained essentially the same in all four subjects throughout the study except for blood urea nitrogen, which decreased significantly soon after the subjects started receiving the experimental diet. This change occurred even before the zinc intake was restricted, suggesting that the decrease in the blood urea nitrogen was due to the change from animal protein to cereal protein in the diet.

In the first two subjects (Patients 1 and 2), who received 2.7 mg of zinc daily, the weight loss was more pronounced in comparison with the second pair of subjects (Patients 3 and 4), who received 3.5 mg of zinc daily. After repletion with zinc, the weight stabilized in three out of four subjects; and in one subject (Patient 1), although the weight loss continued during the zinc-supplemented period, the rate of weight loss as determined by the slope of the curve was decreased.

The changes in body weight correlated highly with the subscapular thickness in the two subjects in whom these data were obtained ($r = 0.839$, $P < 0.001$; $r = 0.938$, $P < 0.001$). Further calculation (14), showed that the weight loss could be

accounted for as follows: 50% fat, 30% water, and 20% other (approximately). In two subjects the intake of fat was 108.3 g/day, and fecal fat (mean ± SD) was 5.6 ± 1.33 g/day in one subject (Patient 4) and in the other 4.11 ± 0.94 g/day throughout the study period. Thus no effect of zinc depletion-repletion was observed on fat balance.

The nitrogen excretion in the feces, urine, and the balance throughout the experimental study period in the second pair of subjects (Patients 3 and 4) showed no remarkable changes. The balance data were apparent balances, inasmuch as the nitrogen excretion in the sweat was not considered in calculating these balances.

Urinary excretion of zinc decreased in three out of four subjects as a result of zinc restriction. In one case (Patient 4) there was no decrease in urinary zinc excretion. This was due to the diuretic therapy (hydrochlorothiazide) that he received for mild hypertension during the study.

In the first two subjects, during the zinc restriction phase (2.7 mg zinc daily intake), the apparent negative balance for zinc ranged from 1 to 4 mg/day, whereas in the second group of subjects the apparent negative balance for zinc was 1 to 2 mg/day. After supplementation with 30 mg of zinc, the positive zinc balance ranged from 11 to 22 mg/day in the first pair of subjects, suggesting a retention of approximately 33% to 70% of zinc intake. In the second pair of subjects (Patients 3 and 4), during the base-line period when the daily zinc intake was 33.5 mg, the positive balance for zinc was 3 to 4 mg daily. On the other hand, these subjects, on the same level of zinc intake (33.5 mg daily) after zinc depletion phase, showed a positive zinc balance of 14 to 16 mg daily.

The plasma zinc level decreased significantly in all 4 subjects as a result of zinc restriction and increased after supplementation with zinc. The changes were more marked from patients on 2.7 mg daily zinc intake compared with those on 3.5 mg daily zinc intake. The erythrocyte zinc level decreased significantly in the first group of two subjects (Patients 1 and 2) although the decrease was not evident until 12 weeks on the restricted zinc intake. In the second group of subjects, although the erythrocyte zinc level did not decrease significantly during the zinc-restricted period, it showed a marked increase after zinc supplementation. Leukocyte zinc decreased significantly due to zinc restriction in the second group of subjects in whom this variable was measured.

Plasma alkaline phosphatase was monitored carefully in the second group of subjects. In both cases, the activity slowly declined as a result of zinc restriction, and after supplementation with zinc, the activity nearly doubled in 8 weeks. In all four subjects, the activity of plasma ribonuclease was almost twice as great during the zinc-restricted period as in the zinc-supplemented phase. Plasma lactic dehydrogenase activity de-

creased with zinc restriction and increased during supplementation phases in two subjects. Plasma ammonia levels were higher during zinc restriction and decreased after zinc supplementation in two subjects (Patients 3 and 4).

In the sponge connective tissue of the first pair of subjects, total protein and total collagen increased significantly during the zinc supplementation phase in comparison with the zinc restriction phase (Patient 1, restriction phase: total protein, 108 mg; total collagen, 29.3 mg; versus supplementation phase: total protein, 226 mg; and total collagen, 58.4 mg; Patient 2, restriction phase: total protein, 94 mg; total collagen 0.013 mg; versus supplementation phase: total protein, 240 mg; total collagen, 121.2 mg). The RNA-DNA ratio in the sponge connective tissue also increased after zinc supplementation (Patient 1, restriction phase: 0.69 versus supplementation phase: 0.82; Patient 2, restriction phase: 0.71 versus supplemental phase: 0.95). There was no detectable activity of deoxythymidine kinase in the sponge connective tissue during the zinc restriction phase, but it was 0.385 units in Patient 1 and 0.321 units in Patient 2 after supplementation with zinc. The activities after supplementation became 70% of the mean normal values (14).

The effect of marginal zinc deficiency on gonadal functions were studied in 5 male volunteers (15). Their ages ranged from 51 to 65 years.

These subjects had normal gonadal function. They had no medical disorder, and they had taken no drugs prior to the studies known to affect testicular function.

Physical examination, routine laboratory tests, and chest roentgenogram were unremarkable. Testicular function as evaluated clinically and by the measurement of serum androgens, FSH, LH, and sperm count were normal in all patients. Zinc status as assessed by the determination of zinc concentration in plasma, erythrocytes, and hair was within normal limits.

During stabilization period, all subjects received hospital diet (10 mg of zinc intake daily). After stabilization, Subject 1 received the experimental diet providing 2.7 mg of daily zinc, with 10 mg of supplemental zinc (as zinc acetate) orally for 6 weeks. Subjects 2 and 3 received the experimentl diet providing 3.5 mg of daily zinc, with 30 mg of supplemental zinc (as zinc acetate) orally for 5 weeks. Subjects 4 and 5 were switched directly from the hospital diet to the experimental zinc-restricted diet. The experimental zinc-restricted diet provided 2.7 mg of daily zinc in Subject 1 for a period of 24 weeks; 3.5 mg of daily zinc in Subjects 2 and 3 for a period of 40 weeks; and 5 mg of daily zinc in Subjects 4 and 5 for a period of 40 and 32 weeks, respectively.

In Subjects 1, 2, and 3, the repletion phase was begun with the administration of 30 mg of zinc (as zinc acetate) orally while the same experimental diet was maintained for a period of

12 weeks in Subject 1 and 8 weeks in Subjects 2 and 3. In Subject 4, 10 mg of zinc (as zinc acetate) was mixed with the experimental diet, for a period of 12 weeks. After the experimental diet, the hospital diet (10 mg of daily zinc) plus the zinc supplementation were given for a period of at least 8 weeks in these four subjects. Subject 5 was not given additional zinc after his experimental diet was terminated but instead received regular diet at home. The calories and protein intake of the hospital and the experimental diet remained the same. Thereafter, all patients received home diet similar to the hospital diet providing 10 mg of daily zinc.

Blood samples were drawn for the determination of LH, FSH, and testosterone before and after stimulation with GnRH. These determinations were performed twice during stabilization period, twice at the end of zinc-depletion period, twice during the first 6 months of zinc-repletion period, and twice after 6 to 12 months of zinc repletion. Thus, the zinc repletion period was divided into two phases: the early phase which reflected the first 6 months of repletion and the late phase which was extended beyond 6 months and up to 12 months of zinc repletion. Blood samples were drawn from an indwelling intravenous catheter between 9:00 and 9:30 a.m. At least two baseline samples 30 min apart were drawn for the determination of serum LH, FSH, and testosterone, and then 200 μg of GnRH were injected intravenously. Blood samples were drawn for the measurement of serum LH and FSH 15, 30, 60, 120, 180, and 240 min later. Blood samples were drawn for the determination of serum testosterone 60, 120, 180 and 240 min after GnRH injection. The baseline value of each hormone was derived by calculating a mean of two to four baseline determinations. Means of two different determinations of GnRH-stimulation tests were calculated for the stabilization, zinc-restriction, and early and late phases of zinc-repletion periods. Thus fluctuation of these hormones from hour to hour, as well as from day to day, was avoided. Serum testosterone, LH, and FSH were measured according to radioimmunoassay techniques.

Semen analysis was done every 4 to 12 weeks and after abstinence from sexual activity for 3 to 7 days before ejaculation. Analysis was done within 30 min of ejaculation. Results of sperm count were expressed in concentration of sperm per milliliter as well as total number of sperm per ejaculate. A mean of two to five sperm counts during each phase of the study were calculated. Oligospermia was defined as a total sperm count less than 40 million per ejaculate.

Zinc concentrations in the erythrocyte and plasma decreased significantly ($p < 0.01$) during zinc restriction in comparison to the stabilization levels. During the early phase of zinc repletion a slight increase in erythrocyte and plasma zinc levels was noted, but these values were not statistically significant in comparison to the zinc-restriction levels. A marked increase in erythrocyte and plasma zinc concentration was observed during the