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Original Research

Zinc Supplementation Overcomes Effects of Copper on Zinc Status, Carbohydrate Metabolism and Some Enzyme Activities in Diabetic and Nondiabetic Rats

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ABSTRACT

Objective: The aim of this study was to investigate the effect of zinc supplementation on zinc status, carbohydrate metabolism and some enzyme activities in rats with alloxan-induced diabetes that were fed high-copper feed.
Methods: Male albino Wistar rats were randomly divided into 6 groups (n=10). The first and fourth groups were nondiabetic and diabetic controls. The second, third, fifth and sixth groups were copper, copper + zinc, diabetes + copper and diabetes + copper + zinc groups, respectively. Diabetes in the fourth, fifth and sixth groups was induced by alloxan. Copper (30 mg/kg feed) as $CuSO_4$ $5H_2O$ and zinc (231 mg/kg feed) as ZnSO4 7H2O were added to the feed of the animals in the copper and zinc groups for 21 days.
Results: Copper supplementation caused a significant decrease in body weight gain, serum zinc, tissue zinc, serum protein concentrations, alkaline phosphatase, lactic dehydrogenase and amylase activities. In contrast, it led to an augmentation in creatinine, uric acid and transaminases activities in rats with and without diabetes. Zinc supplementation in the feed for animals given copper ensured a partial correction of the previous parameters.
Conclusions: The study demonstrated the beneficial effects of zinc treatment in copper-induced metabolic disturbance in diabetic and nondiabetic rats.

Mots clés :
phosphatase alcaline
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RÉSUMÉ

Objectif : Le but de cette étude était d'examiner l'effet de la supplémentation en zinc sur l'état nutritionnel en zinc, le métabolisme des glucides et certaines activités enzymatiques chez les rats ayant un diabète induit par l'alloxane qui ont été nourris avec des aliments riches en cuivre.
Méthodes : Nous avons réparti de manière aléatoire les rats Wistar albinos mâles en 6 groupes (n=10). Les premier et quatrième groupes étaient constitués de témoins non diabétiques et diabétiques. Les deuxième, troisième, cinquième et sixième groupes étaient constitués respectivement comme suit : cuivre, cuivre+zinc, diabète+cuivre et diabète+cuivre+zinc. Le diabète des quatrième, cinquième et sixième groupes était induit par l'alloxane. Nous avons ajouté le cuivre (30 mg/kg de nourriture) sous forme de $CuSO_4$ $5H_2O$ et le zinc (231 mg/kg de nourriture) sous forme de ZnSO4 7H2O à la nourriture des animaux des groupes cuivre et zinc durant 21 jours.
Résultats : La supplémentation en cuivre entraînait une diminution significative du gain de poids corporel, des concentrations sériques et tissulaires de zinc et des concentrations sériques de protéines, et des activités de la phosphatase alcaline, de la lacticodéshydrogénase et de l'amylase. En revanche, elle a entraîné une augmentation de la créatinine, de l'acide urique et des activités des transaminases chez les rats diabétiques ou non. La supplémentation en zinc des aliments des animaux auxquels du cuivre avait été donné assure une correction partielle des paramètres antérieurs.
Conclusions : L'étude a démontré les effets bénéfiques du traitement à base de zinc des troubles du métabolisme induits par le cuivre chez les rats diabétiques et non diabétiques.

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Introduction

Zinc is known to be an essential trace mineral that is necessary for health and growth and is also essential for the function and activity of more than 200 metalloenzymes (1). These enzymes are involved with the metabolism of protein, carbohydrate and lipids (2). Zinc is also required for normal insulin metabolism. Insulin is stored as a hexamer containing 2 zinc ions in pancreas beta cells (3). It seems reasonable, therefore, that changes in body zinc status could affect the production, storage and secretion of insulin (4,5). Diabetes usually leads to hypozincemia and a decrease in tissue zinc stores (6). The possible reason for decreasing serum zinc concentration in patients with diabetes is excessive urinary excretion of zinc (7), so there are several reasons for suspecting that an abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus and some of its complications (8). One of the essential trace elements in living organisms is copper. A great number of biologically active centres contain copper. Copper in the diet is absorbed from the small intestine to the liver. It may be stored within hepatocytes, secreted into plasma or excreted in bile (9). Copper is implicated in the etiology of many neurodegenerative disorders, such as epilepsy and pain (10). This metal is also incorporated into a number of metalloenzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine biosynthesis and the cross-linking of collagen, elastin and hair keratin (11). Its presence in the body combines with enzymes to form metalloenzymes, such as ceruloplasmin and superoxide dismutase (12). It has been postulated that copper possesses insulin-like activity and promotes lipogenesis. One of the most common trace-metal imbalances is elevated copper and depressed zinc. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals (13). Thus, the purpose of this study was to evaluate the effects of zinc supplementation on some parameters in rats with alloxan-induced diabetes that were fed a high copper-content feed.

Methods

Animals and experiment design

Male Wistar albino rats weighing 220 to 280 grams were used in this study. Prior to experiments, rats were allowed to acclimate to their surroundings for 1 week. The animals were housed in individual plastic cages with bedding. Standard rat food (14) and tap water were available ad libitum for the duration of the experiments unless otherwise noted. Trays were placed under each food hopper to collect spilled food. The temperature was maintained at 22°±2°C. A 12/12-hour light/dark cycle was maintained, with lights on at 6 AM unless otherwise noted. Humidity was around 40%. After the successful induction of experimental diabetes, the rats were randomly divided into 6 groups (n=10). The first and fourth groups were nondiabetic and diabetic controls. The second, third, fifth and sixth groups were copper, copper + zinc, diabetes + copper and diabetes + copper + zinc groups, respectively. Diabetes in fourth, fifth and sixth groups was induced by alloxan. Copper (30 mg/kg feed) as $CuSO_4$ $5H_2O$ (15) and zinc (231 mg/kg feed) as ZnSO4 7H2O (16) were added to the animals' feed in the copper and zinc groups. Animals were maintained on the appropriate experimental feed for 21 days. Body weight and food intake were recorded regularly. The Ethical Committee of Annaba University approved the study protocol. The animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by Annaba University.

Induction of diabetes

Diabetes was induced by a fresh alloxan monohydrate solution using a previously described method (17). Alloxan was administered intraperitoneally at a dose of 150 mg/kg body weight dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose was measured 7 days after induction of diabetes in samples taken from the tail vein. The diabetic state was established when the glucose concentration exceeded 14 mmol/L, confirmed by a glucose meter (Accu-Check; Roche Diagnostics, Paris, France).

Blood collection and preparation of blood and tissue samples

On day 22 the rats were killed by cervical cut under ether anesthesia. Two mL of blood were drawn and used for determination of serum zinc, total protein, triglycerides, creatinine, uric acid, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), amylase, lactic dehydrogenase (LDH) and alkaline phosphatase (ALP). The pancreases, kidneys, testes and livers were excised, washed with isotonic saline and blotted to dry. After that, the pancreases, kidneys, testes and livers were weighed and dried at 80°C for 16 hours, and the zinc concentration in each tissue sample was determined.

Measurement of biochemical parameters

The activities of GOT, GPT, ALP, LDH and amylase were determined using commercial kits from Spinreact (Girona, Spain) (refs: GOT-1001161, GPT-1001171, LDH-1001260. ALP-1001131 and amylase-41201). Total protein, creatinine, uric acid and triglyceride concentrations were also measured using commercial kits (Spinreact) (refs: total proteins-1001291, creatinine-1001113, uric acid-1001013 and triglycerides-1001311).

Serum and tissue zinc analyses

Dried pancreases, kidneys, livers and testes were heated in silica crucibles at 480°C for 48 hours, and the ash was dissolved in hot 12 M hydrochloric acid for zinc using a flame atomic absorption spectrophotometer (Shimadzu AA-6200; Somerset, New Jersey, USA). Standard reference materials: bovine liver and wheat flour were used to check the accuracy of zinc recovery, which exceeded 96%. In the serum samples, zinc was determined after 20-fold dilution. In this case, the zinc standards were prepared from a 1 mg/mL zinc nitrate standard solution, using 5% glycerol to approximate the viscosity characteristics and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10% v/v) for 16 hours and rinsed with doubly distilled water (18).

Statistics

Data were reported as mean ± SEM. Results comparisons were carried out by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statically significant at $p<0.05$.

Results

Body weight and food intake

Induction of a type 1 diabetic state caused a decrease ($p<0.001$) in body weight and an increase in food intake in the rats with diabetes compared to the rats without diabetes. Copper also significantly altered ($p<0.001$) body weights of rats without diabetes ($p<0.01$) and rats with diabetes ($p<0.001$), whereas zinc supplementation reversed these changes (Table 1).

Serum and tissue zinc concentrations

Serum zinc, liver zinc and testis zinc contents were significantly lower ($p<0.001$, $p<0.01$) in the group with diabetes than in