FOOD AND NUTRITION

EFFECTS OF ZINC SUPPLEMENTATION ON METABOLIC STATUS IN PATIENTS WITH METABOLIC SYNDROME

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Abstract: Being a micronutrient, zinc involves in numerous biochemical reactions as a principle component or catalyzing enzymes for proper propagation of enzymatic function. Metabolic syndrome (MS) has been increasingly reported to be associated with derangement of the intrinsic physiological functions including suppression of micronutrient functionality and concentration. During the COVID-19 outbreak, a relatively high dose of zinc has been prescribed by private clinics for non-infected/high-risk subjects; who include patients with MS on polytherapy. We aimed to investigate the effect of 50mg-zinc tablet administered as an add-on therapy for three months to patients with MS. According to our health care providers; MS patients undergo an annual quadruple check by measuring the routine biochemical factors (serum glucose, and lipid profile), blood pressure, and body mass indices. Data were collected before and after zinc administration and statistically analyzed in comparison to the control non-zinc user group. The results confirmed that zinc positively improved measured parameters by significantly reducing blood pressure, serum glucose, and lipid indices; together with slightly modulating body mass indices. To confirm that the quality of the zinc tablet provided by the supplier, plasma zinc concentrations were also measured before and after therapy. In conclusion, zinc supplementation could be part of the therapy for metabolic diseases and we do advise intermittent zinc use in such patients.

Keywords: zinc, lipid, hypertension, diabetes, obesity

INTRODUCTION

Micronutrients, including zinc, are essential elements with a pivotal function in human beings. Zinc (Zn) is a trace element that seems to be necessary for a variety of cell functions. Zinc is a vital nutrient that serves as a catalyst for a variety of enzymes and signals transduction, as well as an intrinsic signaling mediator (1). Zinc is required for eyesight, taste perception, cognition, cell reproduction, growth, and immunity, among other functions. Zinc deficiency affects both innate and adaptive immune responses. Oxidative stress, enhanced inflammatory processes, and life-threatening circumstances, as well as accelerated cell death at the cellular and sub-cellular levels, are all signs of zinc deficiency. In previous research, high-dose zinc intake was found to greatly increase patients’ immune systems when they were suffering from viral infections such as torquetenovirus, the common cold, and others (rhinovirus) (2).

More than a hundred catalytic activity Zn metalloproteins and more than 2000 Zn dependent transcription factors were identified, indicating that Zn is a key component of a diverse range of proteins and enzymes, as well as a participant in a broad variety of metabolic methods such as carbohydrate, lipid, protein, and nucleic acid synthesis and destruction. Zn has a wide range of biological roles (3). According to a genome study, approximately 3 and 10% of all target genomes may encode zinc-binding proteins (4). Zinc’s redox persistence (in comparison to other metal complexes) combined with its propensity to form polyhedral coordination compounds with a range of ligands, most prominently histidine and cysteine, makes it a valuable contributor of biological proteins (5). Zinc-containing proteins can be found in all 6 main enzyme categories. The essential methods to preserve zinc homeostasis mostly at the overall organisms and cellular levels are highlighted by these numerous and widespread activities of zinc in biology. Zinc dyshomeostasis appears to play a key role in a variety of crucial pathologies, according to recent research (4).

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As a result, Zinc dysmetabolism, such as Zn deficiency, is linked to a variety of medical conditions. Reaven characterized metabolic syndrome for the first time in 1988 (6), hence it’s also known as Reaven’s syndrome or more commonly metabolic syndrome. Its incidence rises steadily, reaching and over 20% in the adult united states population at the present situation, and it rises with aging (7). Metabolic syndrome is a collection of symptoms that raises the risk of cardiovascular disease and diabetics. In the present study, we have recruited MS patients who have been diagnosed and taking their prolonged medication and using zinc for viral COVID-19 protection and standard biochemical parameters were measured and statistically analyzed to determine the change in the overall outcome.

EXPERIMENTAL

Patients

The age and sex of patients were outlined in Table 1. Consent has been taken from all patients enrolled in this study. Blood pressure (BP), body weight (WT), and west circumferences (WC) were measured for individual subjects, before and after zinc intervention therapy using 50mg daily dose of zinc tablet (ZINC®, Greenfield, USA). Venous blood samples were drawn from all patients and plasma was collected to be frozen for future analysis. The criteria for diagnosis of metabolic syndrome were based on the combined symptoms of; blood pressure (>130/85), plasma triglyceride level (>150 mg/dL), fasting blood glucose level (>100mg/dL), high-density lipoprotein level [<40 mg/dL (men) or <50 mg/dL (women)], and waist circumference [40 inches or more for men and 35 inches or more for women] (8).

Measurement of FBS

Fasting blood sugar (FBS) was determined for all patients using the enzymatic method via Biolabo kit (AT8009, France). The glucose is oxidized to gluconic acid and hydrogen peroxide by the glucose oxidase enzyme. The peroxidase then catalyzes the reaction of peroxide with chlorophe- nol and para-aminonitropyrene resulting in the formation of red-colored quinoneimine, the later complex was quantified at an optical density of 500 nm by spectrophotometry.

Measurement of TC

Serum total cholesterol was determined by the enzymatic method via Biolabo kit (AT80106, France). Cholesterol undergoes 3 consecutive steps catalyzed by cholesterol esterase, cholesterol oxidase, and peroxidase; resulting in subsequent changes in cholesterol structure from cholesterol ester to free form to be converted to cholestenone and finally resulting in the formation of pink-colored quinoneimine, the later complex were quantified at an optical density of 500 nm by spectrophotometry.

Measurement of TG

Serum triglycerides were quantified by the enzymatic method via the Biolabo kit (AT-80019, France). Triglyceride undergoes 4 consecutive steps catalyzed by lipase, glycerol kinase, glycerol 3 phosphate oxidase, and peroxidase; resulting in subsequent changes in the triglyceride structure from triglyceride to glycerol, glycerol 3 phosphate, dihydroxyacetone, and finally pink quinoneimine, the later complex were quantified at an optical density of 500 nm by spectrophotometry.

Measurement of HDL

Serum HDL-cholesterol was measured using a direct method which is an enzymatic colorimetric method. The cholesterol from vLDL, LDL, and chylomicrons was enzymatically released, followed by subsequent enzymatic generation of hydrogen peroxide which was then catalyzed by peroxidase resulting in subsequent conversion into the colorless compound through reaction with parasulphobutylm-toluidine-disodium. This latter compound is a detergent that solubilizes the earlier mentioned lipid particles resulting in the release of HDL. Subsequent steps involved the reaction with cholesterol esterase, cholesterol oxidase, peroxidase; as mentioned earlier in the cholesterol assay step resulting in colored compound formation to be quantified colorimetrically at 600 nm.

Measurement of plasma zinc

Plasma zinc concentration determined by colorimetric methods (Elabscience, E-BC-K137-M, UK) based on the reaction of zinc with 5-Bromo-2-pyridylazo-5-diethylaminophenol forming colored
complex; the intensity of which is proportional to the concentration of zinc in the sample. The color intensity was determined at 560 nm. The sensitivity of the test is that it detects even low concentrations down to 2.7 µg/dL. The comparison was done to confirm that the test is acting properly by comparing to standard serial dilutions of zinc dissolved in distilled water starting from 1 µg/dL up to 10 serial concentrations to reach 512 µg/dL.

**Statistical analysis**

Results are expressed as the mean ± standard deviation (SD). Statistical analysis was done by one-way analysis of variance (ANOVA) using GraphPad Prism V.6, USA. A probability level of $P < 0.05$ was considered significant.

**RESULTS AND DISCUSSION**

Zinc plasma concentration in patients with metabolic syndrome: Zinc administration to a patient with metabolic syndrome has been associated with increased plasma zinc level when compared to before therapy or control group (Figure 1).

Blood pressure changes in patients with metabolic syndrome: Zinc administration to a patient with metabolic syndrome has been associated with a slight reduction of blood pressure plasma zinc level when compared to before therapy or control group (Figure 2).

Body mass parameters in patients with metabolic syndrome: Zinc administration to a patient with metabolic syndrome has been associated with a slight reduction in body mass index when compared to before therapy or the control group (Figure 3).

Metabolic parameters in patients with metabolic syndrome: Zinc administration to a patient with metabolic syndrome has been associated with a significant reduction in metabolic parameters including FBS, TC, TG, and LDL with a non-significant increase in HDL plasma levels when compared to before therapy or control group (Figure 4).

When it comes to the risks and benefits of Zn consumption, it’s pretty safe, though it can cause side effects when eaten in large doses. *In vitro*, unbound
Zn ions caused toxicity in multiple cell lines (3). Poisoning with Zn, on the other hand, has a rather unusual effect on the human body. Recent investigations with consumption considerably well above the maximum limit have yielded conflicting results. For example, taking 60 mg of zinc each day for six weeks had no negative impact on copper condition indices (9). In a 6-month trial, administration of 45 mg Zn/day in the apparently healthy subject led to improved anti-inflammatory and reduced pro-inflammatory factors (10), in this study, we demonstrated that metabolic syndrome patients were shown reduced plasma zinc level, therefore, we use the highest possible dose for correction (50 mg/day, orally)

Our study demonstrated that patients with metabolic syndrome have shown lower zinc concentration than normal healthy individuals and zinc supplementation to these patients shown improved glycemic control and lipid profile together with a slight improvement of blood pressure, Body mass index, and waist circumferences. Lower zinc levels in patients with metabolic syndrome or insulin resistance have been reported earlier by dozens of investigators. Regardless of the efficacy of Zn administration in the treatment or prevention of metabolic syndrome or diabetes, few intervention studies use Zn or with other conjunction with other micronutrients (5, 6-14). No positive changes of Zn administration on any metabolic syndrome factor were seen in four of the ten studies included in Table 2. Four of the other trials show that Zn administration improves both glycemic management and lipid profile.

We confirmed that zinc has slightly improved systolic rather than diastolic blood pressure. The explanation is unclear however NO and oxidative stress could be part of that explanation. As a building element of copper-zinc superoxide dismutase (CuZnSOD)(14), Zn has antioxidant (AOX) properties. CuZnSOD’s regulation of superoxide anion (O2) concentration is crucial in understanding Zn’s preventive function versus high blood pressure. Nitric oxide (NO) is a key indicator of hypertension. NO impacts peripheral permeability by having a significant impact on the vessels by encouraging arterial dilatation. The reduction of sympathetic nerve activity, which is implicated in vascular constriction, is one of NO’s secondary regulation of blood pressure (15). An overabundance of O2 interacts with NO to create peroxynitrite (ONOO), which lowers NO levels (14).

Our results confirmed that zinc has improved the plasma glucose levels resulting in improved glycemic control compared to non-zinc users. The mechanism behind this improvement of zinc on glucose is elusive yet it could be explained in different aspects. In adipose tissue, Zn is implicated in both impaired insulin function. Zn is required for optimal insulin production, crystallization, and development in the pancreatic cell (16). The Zn carrier ZnT8 (SLC30A8) is involved in the entry of Zn ions into secretory vesicles (17). ZnT8 deficiency or deletion induces significant insulin crystals and secretion abnormalities (18). T2D incidence has been linked to the presence of a single-nucleotide variant in ZnT8 (1). Zn preserves insulin-producing tissues from apoptosis mostly via its anti-inflammatory effects (18). Zn reduces IL-1 and TNF-a secretion from phagocytes by inhibiting the expression of the nuclear factor kappa-light-chain amplifier of activated B cells (NF-B) (18). Zn has insulin-mimetic characteristics, which boost the effectiveness of the insulin transmission system (19), resulting in the control of major gluconeogenic enzymes (20). Zn’s insulin-mimetic characteristics are partially controlled by its suppressive

Table 2. Comparative studies in different geography on zinc surplus administration.

| Studies                       | Geography         | Year | Sample size | Duration |
|-------------------------------|-------------------|------|-------------|----------|
| Czernichow S et al. (12)      | France (Europe)   | 2009 | P = 2525, Zn = 2695 | 7.5 years |
| Heidarzadeh Z et al. (11)     | Iran (Asia)       | 2017 | P = 20, Zn = 20  | 6 weeks  |
| Stewart CP et al. (30)        | Nepal (Asia)      | 2009 | P = 735, Zn = 708 | 9 months |
| Mispireta ML et al. (31)      | Perú (América)    | 2017 | P = 86, Zn = 73 | 27 weeks |
| Hashemipour M et al. (32)     | Iran (Asia)       | 2009 | P = 60, Zn = 60  | 8 weeks  |
| Kelishadi R et al. (33)       | Iran (Asia)       | 2010 | P = 60, Zn = 60  | 8 weeks  |
| Lobene AJ et al. (34)         | USA (America)     | 2017 | P = 72, Zn = 75  | 4 weeks  |
| Islam MR et al. (35)          | Bangladesh (Asia) | 2016 | P = 27, Zn = 28  | 6 months |
| Jamilian M et al. (36)        | Iran (Asia)       | 2016 | P = 24, Zn = 24  | 8 weeks  |
| Foroozanfard F et al. (13)    | Iran (Asia)       | 2015 | P = 26, Zn = 26  | 8 weeks  |
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impact on protein-tyrosine phosphatases (PTPs) (21). Zn improves glucose normal metabolic equilibrium regulation by stimulating glucose absorption in insulin-responsive tissues (fatty tissue and muscle) (22). Zn is required for the effective activation of peroxisome proliferator-activated receptors (PPARs), which can lead to the production of the GLUT genes (11). In addition to pancreatic islets, Zn is found in the liver. Zn reduces glucagon release by adhering to and activating ATP-responsive potassium signaling pathways, desensitizing the cells, and blocking voltage-sensitive calcium channel stimulation (23).

The outcome of our investigations confirmed that zinc played a role in the reduction of lipid profile indicated by reduced total cholesterol and triglyceride level plus upregulation of HDL profile. Pancreatic A2 phospholipase (PLA2), a protein responsive to Zn deficiency, Zn enhances gastric absorption of the essential fatty acids, alpha-linolenic acid (ALA), and linoleic acid (LA) (24). Zn also acts as a coenzyme for the delta 6 desaturase enzyme, which promotes the conversion of ALA and LA from the dietary to polyunsaturated fatty acids (25). PUFA insufficiency has been associated with increases in triacylglyceride (TAG) released by the liver and, as a result, hypertriglyceridemia (26). As a result, sufficient Zn concentrations are needed to avoid an atherogenic lipid panel. Zn also plays a role in fatty acid formation, assembly, and removal in lipid biomolecules by activating PPARs (27). The genetic traits that translate for apoprotein A-I and apoprotein A-II, which have been essential of HDL, lipoprotein lipase, and ATP-binding cassette transporter A1, amongst others, are all substrates of PPARs (28). PPAR stimulation has been proposed as a defense effect towards coronary artery disease. In terms of lipolysis, Zn works as a potent inhibitor by blocking adipose tissue lipolysis generated by - adrenergic receptor stimuli by inhibiting PTPs, phosphodiesterase stimulation, and, as a result, hormone-sensitive lipase (HSL) inactivation via its insulin-mimetic characteristics (29).

CONCLUSIONS

Zinc administration has been associated with improved overall measured parameters for patients with metabolic syndrome including improvement in systolic blood pressure, body mass indices, and metabolic parameters; hence, zinc deficiency or zinc status correction should be advised in patients with metabolic syndrome.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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