HORMONES 2009, 8(4):279-285

Research paper

Effect of zinc supplementation on insulin resistance and components of the metabolic syndrome in prepubertal obese children

Mahin Hashemipour[1], Roya Kelishadi[2], Javad Shapouri[1], Nizal Sarrafzadegan[2], Masoud Amini[1], Naser Tavakoli[3], Ahmad Movahedian-Attar[3], Parisa Mirmoghtadaee[3], Parinaz Poursafa[4]

[1]Isfahan Endocrine & Metabolism Research Center and [2]Isfahan Cardiovascular Research Center and [3]Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Science & Research University, Tehran, Iran

ABSTRACT

OBJECTIVE: Zinc, an essential trace element and a component of many enzymes, is involved in the synthesis, storage and release of insulin. The aim of the present study was to assess the effect of zinc supplementation on insulin resistance and components of the metabolic syndrome in prepubertal obese children. DESIGN: This triple-masked, randomized, placebo-controlled cross-over trial was conducted among 60 obese Iranian children in 2008. Pertinent clinical findings, fasting serum glucose, insulin and lipid profile were assessed. Participants were randomly assigned to two groups of equal number; one group received 20mg elemental zinc and the other group received placebo on a regular daily basis for eight weeks. After a 4-week wash-out period, the groups were crossed over. RESULTS: The mean age of participants was 9.1 ± 1.1 years. After receiving zinc, the mean fasting plasma glucose (FPG), insulin and HOMA-IR decreased significantly, while body mass index (BMI), waist circumference (WC), LDL-C and triglycerides (TG) did not significantly change. After receiving placebo, the mean FPG, insulin and HOMA-IR increased significantly, while BMI, WC, LDL-C and TG showed a non-significant increase. CONCLUSION: Besides lifestyle modification, zinc supplementation might be considered as a useful and safe additional intervention treatment for improvement of cardiometabolic risk factors related to childhood obesity.

Key words: Cardiometabolic risk factors, Childhood obesity, Metabolic Syndrome, Pre-pubertal stage, Zinc

Address for correspondence:
Roya Kelishadi, MD, Associate Professor of Pediatrics, Head, Pediatric Preventive Cardiology Department, Isfahan Cardiovascular Research Center, Isfahan University of Medical Sciences, P.O. Box 81465-1148, Isfahan, Iran, Tel.: +98 311 3377881-8, Fax: +98 311 3373435, e-mails: kroya@aap.net , Kelishadi@med.mui.ac.ir

Received 15-04-09, Revised 20-08-09, Accepted 15-09-09

INTRODUCTION

Low- and middle-income countries are facing a double burden of nutritional disorders, i.e. malnutrition and micronutrient deficiencies as well as a rapidly growing epidemic of childhood obesity.[2]

The rise in the incidence of childhood obesity significantly increases the number of new cases of metabolic syndrome (MetS) among children.[3] Moreover, the problem is not limited to industrialized countries, since the Middle East is one of the regions with the highest prevalence of childhood obesity and MetS because of rapid lifestyle change and the still existing belief among families that childhood overweight is a sign of health.[4] Given that the Middle Eastern population is facing the world's greatest increment in the absolute burden of future diabetes,[5] preventive measures from early life are imperative.

It is therefore extremely important to try to prevent or treat MetS via simple and feasible methods. Healthy lifestyle remains the first-line intervention for its prevention and control in all age groups.[3] However, some pharmacologic approaches can also be considered.

Regardless of the existing controversies, insulin resistance is considered as a generally accepted unifying theory explaining the pathophysiology of MetS.[6] Thus, it could be suggested that factors related to the insulin metabolism might improve insulin resistance and consequently the components of MetS.

Zinc, an essential trace element and a component of many enzymes, is involved in the synthesis, storage and release of insulin. Many experimental and clinical studies have documented the fact that zinc deficiency may predispose to glucose intolerance, diabetes mellitus, insulin resistance, atherosclerosis and coronary artery disease.[7-9] The effect of zinc on low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) has also been reported.[7,10]

Unlike the effects of macronutrients on MetS which have been extensively investigated,[1,10,11] the potential effects of micronutrients have received limited attention and such studies are particularly sparse in the pediatric age group.

In the present study, we evaluated the effect of zinc sulfate on insulin resistance and components of the metabolic syndrome in a sample of obese children. Because of the hormonal changes during puberty, the trial was conducted among prepubertal children.

METHODOLOGY

Participants

This triple-masked, randomized, placebo-controlled cross-over trial was conducted among 60 obese Iranian children in 2008. Participants were randomly recruited from among 97 obese children aged 6-10 years who were referred from January to March 2008 to the Pediatric Obesity and Metabolic Syndrome Research Clinic of the Pediatric Preventive Cardiology Department, Isfahan Cardiovascular Research Center (ICRC), a collaborating center of the World Health Organization. Participants were selected by means of a computer-generated random numbers table by using the children's records numbers in our clinic. The parents of seven selected children did not agree to have their children included in this trial, hence the recruitment was continued to reach the required number of 60 participants.

The study was approved by the Ethics Committee of the ICRC (NIH Code: FWA 0000t8578) and was conducted according to the Declaration of Helsinki. After providing detailed oral information to children and parents, we obtained written informed consent from the parents and oral assent from the children.

Eligibility criteria for participation included age between 6 and 10 years, body mass index (BMI) equal to or higher than the age- and sex-specific 95th percentile according to the revised Centers for Disease Control and Prevention (CDC) growth charts[12] and no signs of puberty (Tanner stage 1).[13] Children with syndromal obesity, endocrine disorders, any physical disability, history of chronic medication use, use of mineral and/or vitamin supplements, history of any chronic diseases and/or chronic medication use or children under special diets were not included in the study.

Anthropometric Measurement and Clinical Examination

The age and birth date of subjects were recorded. All anthropometric measurements were made by the

same trained general physician and under the supervision of the same pediatrician following standard protocols.

Height (Ht) and weight (Wt) were measured twice to ±0.2 cm and to ±0.2 kg, respectively, with subjects being barefoot and lightly dressed; the averages of these measurements were recorded. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Waist circumference (WC) was measured with a non-elastic tape at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration.

Blood pressure (BP) was measured using mercury sphygmomanometers after 5 min of rest in the sitting position. The subjects were seated with the heart, cuff, and zero-indicator on the manometer at the level of the observer's eye. All readings were taken in duplicate in the right arm. Appropriate size cuffs were used with cuff-width 40% of mid-arm circumference, and cuff bladders covering 80% to 100% of the arm circumference and approximately two thirds of the length of the upper arm without overlapping. The procedure was explained to the children and the cuff inflated and deflated once, the first BP measured was not used in the analysis of this study. The readings at the first and the fifth Korotkoff phase were taken as systolic and diastolic BP (SBP and DBP), respectively. The average of the two BP measurements was recorded and included in the analysis.[14] A pediatric endocrinologist determined the pubertal developmental stage by careful physical examination according to the Marshall and Tanner score.[13]

Biochemical measurements

The biochemical parameters were measured in a central laboratory of the ICRC with adherence to external national and international quality control. The children were instructed to fast for 12 h before the screening; compliance with fasting was determined by interview on the morning of examination. With one of the parents accompanying his/her child, blood samples were taken from the antecubital vein between 08:00 and 09:30 am. After collecting blood samples, the participants were served a healthy snack provided by the project team. In addition to the components of the metabolic syndrome, total cholesterol (TC) and LDL-C were measured as well. Fasting plasma glucose (FPG), triglycerides (TG), TC, LDL-C and high-density lipoprotein-cholesterol (HDL-C) were measured enzymatically by auto-analyzer (Hitachi, Tokyo, Japan).

Insulin was measured by the immune radiometric assay (IRMA) method (Biosource, Belgium). Insulin resistance (IR) was calculated by the homeostasis model assessment for insulin resistance (HOMA-IR) model as fasting $\text{insulin}_{(\text{mU/L})}$ x fasting $\text{glucose}_{(\text{mmol/L})}$/ 22.5.

Serum zinc level was measured by flame atomic absorption spectrophotometry in the Biochemistry Department of the Faculty of Pharmacy, Isfahan University of Medical Sciences.

Intervention

After baseline measurements, participants were randomly assigned into two groups of equal number by means of a computer-generated random numbers table by using the children's records numbers in our clinic.

There is no documented dose of zinc supplementation for improvement of insulin resistance among obese children. As trials among adults have revealed favorable changes with a dose of 30mg/day of elemental zinc,[15,16] that is, two times higher than the recommended dietary allowance (RDA) for adults of 15mg/day, [17] we used a dose of 20mg/day of elemental zinc, i.e. two-fold the RDA for children.[17] One group received 20mg elemental zinc and the other group received placebo on a regular daily basis for eight weeks. The trial was conducted according to a triple blind randomized method, i.e. the physician who prescribed the drug, the nurse of our clinic who gave the drug and the participants and their families were not aware of the type of drug used. Since the groups were coded as Group 1 and 2, the statistician who analyzed the data was not aware of the intervention provided for each group. The drugs and placebo were of the same size and color and were prepared in the Pharmaceutics' Department of the Faculty of Pharmacy, Isfahan University of Medical Sciences.

In order to increase the participants' compliance and to identify possible side effects, all participants and their parents were followed up via a weekly telephone call and a monthly visit during the trial. The

baseline clinical and laboratory measurements were repeated eight weeks after starting the treatment (zinc or placebo). Then, after a 4-week wash-out period, the groups were crossed over so that the children initially receiving zinc received placebo and children previously taking the placebo received 20mg elemental zinc per day. The protocol was repeated again for eight weeks. At the end of the second phase, all laboratory tests and clinical evaluations were repeated. The whole program was offered free of charge.

Statistical analysis

We used the SPSS for Windows software (version 15:00; SPSS, Chicago, IL) for statistical analysis. Descriptive data are expressed as mean ± standard deviation (SD). After assessment of the normal distribution by the Kolmogorov-Smirnov test, within group changes were compared by the paired t-test for those variables with normal distribution and by the Wilcoxon Signed Rank test for SBP, DBP, serum zinc and insulin levels as well as HOMA-IR that had non-normal distribution. For comparison of data between the two groups, we used Student's *t*-test for data with normal distribution and the Wilcoxon Signed Rank test for the aforementioned data concerning non-normal distribution.

Analyses were initially stratified by gender, but as the differences were not significant, results are presented for girls and boys combined. A P value of less than 0.05 was considered as statistically significant.

RESULTS

The trial recorded no drop-outs and all 60 children (59% females) completed the trial. The mean age of participants was 9.1±1.1 years. The two groups under study had no significant difference in terms of age, the male/female proportion and other baseline characteristics. All participants lived in Isfahan city and were generally from middle-income families.

Mean (SD) of anthropometric measures, BP, serum insulin, FPG and lipid profile as well as HOMA-IR before and after receiving zinc supplement or placebo are presented in Tables 1 and 2. In both groups, the changes in weight, BMI, BMI *Z*-score, total- and LDL-cholesterol, FPG, insulin and HOMA-IR were significantly different after receiving zinc supplement vs. placebo. In both groups, the mean BMI *Z*-score remained high; however, after zinc supplementation, the mean weight, BMI, BMI *Z*-score, total- and LDL-cholesterol, FPG, insulin and HOMA-IR

Table 1. Characteristics (mean ± SD) of children receiving zinc supplement followed by placebo.

	Zinc		Placebo		
	Before	**After**	**Before**	**After**	**P value***
Weight (kg)	45.63±10.14¶	43.91±10.03¶	44.05±9.65‡	46.68±10.02‡	0.01
Height (cm)	135.61±7.57	137.00±7.41	137.60±7.62	138.16±7.45	0.07
Body mass index (kg/m^2)	25.05±3.70¶	23.29±3.64¶	23.21±3.28‡	24.87±3.40‡	0.01
Body mass index-Z score (SD)	3±0.41¶	2.5±0.42¶	2.5±0.37‡	3±0.45‡	0.02
Waist circumference (cm)	82.36±8.60	81.93±9.00	82.92±6.56	83.26±7.11	0.25
Systolic blood pressure (mmHg)	95.89±10.00	96.88±10.30	96.12±10.79	94.21±10.12	0.23
Diastolic blood pressure (mmHg)	54.46±8.75	54.17±6.54	54.76±8.47	54.00±10.38	0.17
Serum zinc (µmol/L)	11.88±4.36¶	13.47±2.24¶	12.97±3.43‡	11.48±3.39‡	0.03
Total cholesterol (mmol/L)	4.68±0.71¶	4.58±0.69¶	4.61±0.69	4.66±0.67	0.01
LDL-C (mmol/L)	2.87±0.67¶	2.65±0.44¶	2.64±0.59	2.75±0.55	0.03
HDL-C (mmol/L)	1.13±0.23	1.18±0.24	1.18±0.34	1.11±10.12	0.40
Triglycerides (mmol/L)	1.46±0.24¶	1.37±0.25¶	1.39±0.22‡	1.49±0.29‡	0.051
Fasting plasma glucose (mmol/L)	4.81±0.53¶	4.48±0.32¶	4.43±0.45‡	4.69±0.46‡	<0.0001
Insulin (mU/dL)	21.38±9.97¶	16.51±7.75¶	17.12±6.92‡	19.65±8.53‡	0.02
HOMA-IR	4.75±1.46¶	3.26±1.57¶	3.27±1.62‡	4.19±1.05‡	0.01

*: P value of variables after receiving zinc supplement *vs.* placebo obtained by Student's *t*-test except for systolic and diastolic blood pressures, zinc, insulin and HOMA-IR with non-normal distribution tested by Wilcoxon Signed Rank test
¶: $p<0.05$ after vs. before receiving zinc; ‡: $p<0.05$ after vs. before receiving placebo

Table 2. Characteristics (mean± SD) of children receiving placebo followed by zinc supplement.

	Placebo		Zinc		
	Before	After	Before	After	P value*
Weight (kg)	45.47±11.00‡	47.82±11.28‡	47.51±11.56¶	45.26±10.30¶	0.01
Height (cm)	136.61±7.57	137.00±7.41	137.60±7.62	137.96±7.45	0.07
Body mass index (kg/m^2)	24.71±3.70‡	25.19±3.64‡	25.28±3.27¶	23.75±3.40¶	0.03
Body mass index-Z score (SD)	3±0.38	3±0.46	3±0.42	2.5±0.37	0.01
Waist circumference (cm)	82.36±8.60	82.93±9.00	82.92±6.56	81.26±7.11	0.25
Systolic blood pressure (mmHg)	95.89±10.00	96.88±10.30	95.12±10.79	93.21±10.12	0.23
Diastolic blood pressure (mmHg)	54.46±8.75	54.17±6.54	54.76±8.47	50.00±10.38	0.17
Serum zinc (µmol/L)	11.88±4.36	11.94±3.24	11.94±3.39¶	12.99±3.43¶	0.03
Total cholesterol (mmol/L)	4.69±0.71	4.66±0.69	4.63±0.69¶	4.48±0.97¶	0.01
LDL-C (mmol/L)	2.82±0.67	2.85±0.44	2.85±0.52¶	2.68±0.55¶	0.03
HDL-C (mmol/L)	1.19±0.23	1.13±0.24	1.10±0.34	1.11±0.26	0.40
Triglycerides (mmol/L)	1.43±0.24‡	1.48±0.25‡	1.49±0.22¶	1.41±0.29¶	0.01
Fasting plasma glucose (mmol/L)	4.70±0.53	4.81±0.32	4.80±0.44¶	4.43±0.45¶	0.001
Insulin (mU/dL)	21.12±3.75‡	22.75±3.77‡	22.38±9.97¶	19.51±7.75¶	0.03
HOMA-IR	4.12±1.79‡	4.87±1.54‡	4.85±1.62¶	3.91±1.54¶	0.02

*: P value of variables after receiving zinc supplement *vs.* placebo obtained by Student's *t*-test except for systolic and diastolic blood pressures, zinc, insulin and HOMA-IR with non-normal distribution tested by Wilcoxon Signed Rank test
¶: $p<0.05$ after vs. before receiving zinc; ‡: $p<0.05$ after vs. before receiving placebo

decreased significantly. After placebo, the mean weight, BMI, BMI *Z*-score, TG, FPG, insulin and HOMA-IR increased significantly, and the mean WC, total- and LDL-cholesterol showed a non-significant increase.

DISCUSSION

To the best of our knowledge, the data obtained in this study on the effects of zinc on insulin resistance and the components of the metabolic syndrome in prepubertal obese children constitute a novel observation. We found that 20mg daily elemental zinc supplementation was effective in reducing insulin resistance. Children receiving zinc supplementation showed significant favorable changes in BMI, some cardiometabolic risk factors and insulin resistance, whereas the corresponding figures were not improved after receiving placebo. Such an effect of zinc may prove of clinical significance.

The effect of zinc on inhibition of glycogen synthase kinase 3β has been shown in laboratory studies.[18] In a trial among diabetic patients, zinc supplementation was effective in reducing HbA_1C.[7] Furthermore, some studies have suggested that zinc supplementation could improve insulin sensitivity in patients with non-insulin-dependent diabetes mellitus.[19]

The national prevalence of MetS in Iran is high, and is estimated to be 34.7% among adults[20] and 14.2% among children and adolescents.[21] Therefore, zinc supplementation or increasing zinc intake through diet might be an additional means of reducing the hazards of this emerging health problem and its consequences.

In the present trial, the mean total- and LDL-cholesterol as well as TG decreased significantly after the subjects received zinc supplement. The majority of controlled trials which evaluated the effect of zinc supplementation on plasma lipids did not document significant changes on serum lipids with 15-150mg daily zinc.[22-24] On the other hand, it was shown that supplementation with 20 or 53mg zinc/day can decrease plasma cholesterol concentrations in older people with low zinc serum level.[22,25] Such changes were not found for serum TG levels.[22,23] The non-significant change in HDL-C in this study was consistent with other studies. The effect of zinc supplementation in males appears to be dependent on the dose of zinc and the duration of the supplementation. Doses of

30mg zinc/day for up to 14 weeks had no significant effect on HDL-C concentrations, while higher doses decreased it.[22,24] The favorable changes in serum lipid profile after zinc supplementation in the present trial may be attributed to the decline in BMI.

Our finding of a significant decline in BMI after zinc intake is in line with previous studies among adults in which a beneficial effect of daily zinc intake on the body fat of obese individuals was demonstrated.[10,25]

A dose-dependent rise of plasma rennin activity and serum aldosterone levels, without significant changes in blood pressure, was observed in normotensive individuals undergoing additional zinc supplementation.[26] We did not observe any significant change in blood pressure, a finding which is in line with a study among diabetic patients with microalbuminuria.[16]

The main limitation of the present study might be its short time of follow-up, since it is probable that changes in some anthropometric measures and cardiometabolic risk factors could reach significance with longer follow-up. The other limitation concerns the dose of zinc used in this trial: as there was no documented dose of zinc supplementation for improvement of insulin resistance among obese children, we estimated a dose based on trials among adults. Although the dose used in this trial was high, we did not document any side effect; this might in part be attributed to the high prevalence of zinc deficiency in our community. Future studies should compare different doses of zinc supplementation for children. Moreover, the time for the wash-out period, adopted from studies conducted in adults, might have been shorter than required, a fact that possibly explains the rather high basal zinc values in children given the placebo after the zinc. The novelty of this study is its inclusion of prepubertal children. The strength of the trial was its cross-over design that overcome many confounding factors like diet and physical activity habits among participants; moreover, its triple-masked, placebo-controlled method increases the validity of the findings.

CONCLUSION

Besides application of lifestyle modifications, zinc supplementation might be considered a useful and safe additional intervention for controlling cardiometabolic risk factors related to childhood obesity. Our findings on the efficacy and safety of zinc supplementation should be confirmed by future studies with a larger sample size and a longer follow-up period.

Acknowledgments: This trial was funded by the Vice-Chancellery for Research, Isfahan University of Medical Sciences, Isfahan, Iran

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