

# Effect of Zinc Supplementation on Markers of Insulin Resistance, Oxidative Stress, and Inflammation among Prepubescent Children with Metabolic Syndrome

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## Abstract

**Objective:** This trial aimed to evaluate the effects of zinc sulfate in comparison with placebo on markers of insulin resistance, oxidative stress, and inflammation in a sample of obese prepubescent children.

**Methods:** This triple-masked, randomized, placebo-controlled, crossover trial was conducted among 60 obese Iranian children in 2008. Participants were randomly assigned to two groups of equal number; one group received 20 mg of elemental zinc and the other group received placebo on a regular daily basis for 8 weeks. After a 4-week washout period, the groups were crossed over. In addition to anthropometric measures and blood pressure, fasting plasma glucose, lipid profile, insulin, apolipoproteins A-1 (ApoA-I) and B, high-sensitivity C-reactive protein (hs-CRP), leptin, oxidized low-density lipoprotein (ox-LDL), and malondialdehyde were determined at all four stages of the study.

**Results:** Irrespective of the order of receiving zinc and placebo, in both groups, significant decrease was documented for Apo B/ApoA-I ratio, ox-LDL, leptin and malondialdehyde, total and LDL-cholesterol after receiving zinc without significant change after receiving placebo. In groups, hs-CRP and markers of insulin resistance decreased significantly after receiving zinc, but increased after receiving placebo. In both groups, the mean body mass index (BMI) Z-score remained high, after receiving zinc, the mean weight, BMI, BMI Z-score decreased significantly, whereas these values increased after receiving placebo.

**Conclusion:** These results are particularly important in light of the deleterious consequences of childhood obesity and early changes in markers of inflammatory and oxidative stress. We suggest exploring the direct clinical application of zinc supplementation in childhood obesity in future studies.

## Introduction

THE PROCESS OF MANY chronic diseases starts during fetal life, and the natural course consists of interrelations of traditional risk factors with inflammatory, immune, and oxidative biomarkers.<sup>1</sup> The rapidly escalating trend of childhood obesity is becoming a potential emerging global health concern with long-term health impact, notably on

chronic noncommunicable diseases (CNCD).<sup>2</sup> Insulin resistance, inflammation, and oxidative stress are interrelated<sup>3</sup> and might be associated with enhanced free radical production and in turn with harmful health effects. The presence of such markers has been documented in childhood and is more prevalent in those that are overweight and obese.<sup>4–6</sup> Given that earlier prevention and control of risk factors of chronic diseases would have better results,<sup>7</sup> it is important to

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find simple and feasible methods for prevention and control of factors accelerating the underlying process. In this regard, some pharmacologic approaches might be useful to be considered in addition to healthy lifestyle, which is the first-line intervention.

Antioxidant treatments, such as vitamin E,<sup>8</sup> vitamin C,<sup>9</sup> and lipoic acid<sup>10</sup> have been shown to be capable of improving insulin function. Zinc is an essential trace element and a component of many enzymes; it is involved in the synthesis, storage, and release of insulin. It has been documented that zinc deficiency may be a predisposing factor for insulin resistance, glucose intolerance, and diabetes mellitus, as well as atherosclerosis and coronary artery disease.<sup>11,12</sup> Treatment of zinc deficiency in patients with type 1 diabetes mellitus is found to be effective in improvement in glucose homeostasis, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), as well as in decreasing lipid peroxidation.<sup>13–15</sup>

Considering the possible favorable effects of zinc on insulin sensitivity and its antioxidant functions, along with the lack of such studies in the pediatric age group, this trial aimed to evaluate the effects of zinc sulfate in comparison with placebo on markers of insulin resistance, oxidative stress, and inflammation in a sample of obese children.

## Methods

Detailed methods have been previously described.<sup>16</sup> Here we present a brief description of the methods with particular focus on biochemical tests not described before.

### Participants

This triple-masked, randomized, placebo-controlled, crossover trial was conducted among 60 obese Iranian children in 2008. Participants were randomly recruited from among 97 obese children aged 6–10 years old, 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.

The study was approved by the Ethics Committee of ICRC [National Institutes of Health (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 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 95<sup>th</sup> percentile according to the revised Centers for Disease Control and Prevention (CDC) growth charts,<sup>17</sup> and being in the prepubertal stage (Tanner stage 1).<sup>18</sup> Children with syndromal obesity, endocrine disorders, any physical disability, history of chronic medication use, using mineral and/or vitamin supplements, history of any acute infection, chronic diseases, and/or chronic medication use as well as those under special diet were not included to the study.

### Physical examination

All measurements were made by the same trained general physician and under the supervision of the same pediatrician

by following standard protocols. Height and weight were measured, and BMI was calculated as weight (kg) divided by the square of height (m<sup>2</sup>). Waist circumference (WC) was measured with a nonelastic 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 was measured twice using mercury sphygmomanometers after 5 min of rest in the sitting position. The readings at the first and the fifth Korotkoff phase were taken as systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. The average of the two blood pressure measurements was recorded and included in the analysis.<sup>19</sup>

A pediatric endocrinologist determined the pubertal developmental stage by careful physical examination according to Marshall and Tanner score.<sup>18</sup>

### Biochemical measurements

The children were instructed to fast for 12 h before the screening; compliance with fasting was determined by interview on the morning of examination. Serum zinc level was measured by flame atomic absorption spectrophotometry in the Biochemistry Department of the Faculty of Pharmacy, Isfahan University of Medical Sciences. Other biochemical parameters were measured in central laboratory of ICRC with adherence to external national and international quality control.

Fasting plasma glucose (FPG) and lipid profile, including total cholesterol (TC), LDL-C, HDL-C, and triglycerides (TG) were measured enzymatically (Pars Azmoun, Tehran, Iran) by autoanalyzer (Hitachi, Tokyo, Japan). Apolipoproteins A-1 (ApoA-1) and ApoB were measured using immunoturbidimetry by the same auto analyzer. High-sensitivity C-reactive protein (hs-CRP) was measured by the same autoanalyzer. Serum leptin was measured with the enzyme-linked immunosorbent assay (ELISA) method using the direct DBC kit (Diagnostic Biochem Canada). Insulin level was measured by immunoradiometric assay (IRMA) method (Biosource, Belgium). Insulin resistance (IR) was calculated by homeostasis model assessment for insulin resistance (HOMA-IR) model as  $[\text{fasting insulin}_{(\text{mU/L})} \times \text{fasting glucose}_{(\text{mmol/L})} / 22.5]$ .

The concentration of oxidized (ox)-LDL in plasma was measured with an ELISA procedure by using the murine monoclonal antibody monoclonal antibody (mAb) 4E6 as capture antibody and a peroxidase-conjugated anti-apolipoprotein B antibody recognizing ox-LDL bound to the solid phase (Mercodia AB, Uppsala, Sweden). The measurement of malondialdehyde (MDA) was performed as described by Eslerdauer et al.<sup>20</sup>

### Intervention

After baseline assessment, children were randomly assigned into two groups of equal number by means of computer-generated random numbers table by using the children's records numbers in our clinic. Given that there was no documented dose of zinc supplementation for improving insulin resistance among obese children, and trials among adults revealed favorable changes with a dose of 30 mg/day of elemental zinc,<sup>14,21</sup> which is two times higher than the recommended dietary allowance (RDA) for adults,

i.e., 15 mg/day, we used a dose of 20 mg/day of elemental zinc, i.e., two-fold of RDA for children.<sup>16</sup>

One group received 20 mg of elemental zinc, and the other group received placebo on a regular daily basis for 8 weeks. The drug and placebo had the same size and color and were prepared in the Pharmaceutics' Department of the Faculty of Pharmacy, Isfahan University of Medical Sciences. The trial was conducted in a triple-masked, 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 the medication used. Moreover, as groups were coded, the statistician who analyzed the data could not recognize the two groups and was not aware of the intervention provided for each group.

All participants and their parents were followed up by weekly telephone call and monthly visit during the trial. All baseline measurements were repeated 8 weeks after starting the treatment (zinc or placebo). Then, after a 4-week washout period, the groups were crossed over, so that the children initially receiving zinc received the placebo and children previously taking the placebo received 20 mg of elemental zinc per day. The protocol was repeated again for 8 weeks. At the end of the study, all laboratory tests and clinical evaluations were repeated. The whole program was offered free of charge.

### Statistical analysis

Descriptive data were expressed as mean  $\pm$  standard deviation (SD). After assessment of the normal distribution by the Kolmogorov-Smirnov test, within-group changes were compared by paired *t*-test for those variables with normal

distribution, and by Wilcoxon signed ranks test for SBP, DBP, serum zinc insulin, and CRP levels as well as HOMA-IR that had nonnormal distribution. For comparison of data between the two groups, we used the Student *t*-test for data with normal distribution and Wilcoxon signed ranks test for those data with nonnormal distribution.

Analyses were initially stratified by gender, but because the differences were not significant, results are presented for girls and boys combined. We used the SPSS for Windows software (version 15.00; SPSS, Chicago, IL) for statistical analysis. The significance level was set at *P* value of less than 0.05.

### Results

The trial had no dropouts, and all 60 children (59% females) completed the trial. The mean age of participants was  $9.1 \pm 1.1$  years. The two groups under study had no significant difference in terms of age group and the male/female proportion. The age range was 6 years 8 months to 10 years 5 months in the first group, and 6 years 10 months to 10 years 4 months in the second group.

The means (SD) of parameters studied before and after receiving zinc supplement and placebo are presented in Tables 1 and 2. In both groups, the mean BMI Z-score remained high after receiving zinc; the mean weight, BMI, and BMI Z-score decreased significantly; and these values increased after receiving placebo. Irrespective of the order of receiving zinc and placebo, in both groups, significant decrease was documented for the Apo B/ApoA-I ratio, ox-LDL, leptin and malondialdehyde, total and LDL-C after receiving zinc without significant change after receiving placebo. In both groups, hs-CRP and markers of insulin resistance decreased significantly after receiving zinc, but increased after receiving placebo.

TABLE 1. CHARACTERISTICS (MEAN  $\pm$  STANDARD DEVIATION) OF CHILDREN RECEIVING ZINC SUPPLEMENTATION FOLLOWED BY PLACEBO

	Zinc		Placebo		P value <sup>a</sup>
	Before	After	Before	After	
Weight, kg	45.63 $\pm$ 10.14 <sup>b</sup>	43.91 $\pm$ 10.03 <sup>b</sup>	44.05 $\pm$ 9.65 <sup>c</sup>	46.68 $\pm$ 10.02 <sup>c</sup>	0.01
Body mass index, kg/m <sup>2</sup>	25.05 $\pm$ 3.70 <sup>b</sup>	23.29 $\pm$ 3.64 <sup>b</sup>	23.21 $\pm$ 3.28 <sup>c</sup>	24.87 $\pm$ 3.40 <sup>c</sup>	0.01
Body mass index Z-score, SD	3 $\pm$ 0.41 <sup>b</sup>	2.5 $\pm$ 0.42 <sup>b</sup>	2.5 $\pm$ 0.37 <sup>c</sup>	3 $\pm$ 0.45 <sup>c</sup>	0.02
Waist circumference, cm	82.36 $\pm$ 8.60	81.93 $\pm$ 9.00	82.92 $\pm$ 6.56	83.26 $\pm$ 7.11	0.25
Serum zinc, mg/dL	77.68 $\pm$ 28.53 <sup>b</sup>	88.09 $\pm$ 21.17 <sup>b</sup>	85.00 $\pm$ 22.43 <sup>c</sup>	75.08 $\pm$ 22.19 <sup>c</sup>	0.03
Total cholesterol, mg/dL	180.36 $\pm$ 27.18 <sup>b</sup>	176.14 $\pm$ 26.43 <sup>b</sup>	177.18 $\pm$ 26.57	179.37 $\pm$ 25.62	0.01
LDL-C, mg/dL	110.36 $\pm$ 25.85 <sup>b</sup>	101.79 $\pm$ 16.80 <sup>b</sup>	101.68 $\pm$ 19.95	105.95 $\pm$ 21.20	0.03
HDL-C, mg/dL	43.68 $\pm$ 9.01	45.54 $\pm$ 9.23	45.45 $\pm$ 13.02	42.84 $\pm$ 10.12	0.40
Triglycerides, mg/dL	132.43 $\pm$ 21.95 <sup>b</sup>	124.39 $\pm$ 22.85 <sup>b</sup>	126.45 $\pm$ 20.09 <sup>c</sup>	135.57 $\pm$ 26.01 <sup>c</sup>	0.051
Apolipoprotein A-I, mg/dL	135.4 $\pm$ 12.7 <sup>b</sup>	139.1 $\pm$ 14.4 <sup>b</sup>	137.1 $\pm$ 11.4	135.1 $\pm$ 12.4	0.03
Apolipoprotein B, mg/dL	72.4 $\pm$ 8.1 <sup>b</sup>	67.5 $\pm$ 7.1 <sup>b</sup>	68.7 $\pm$ 7.2 <sup>c</sup>	71.5 $\pm$ 7.5 <sup>c</sup>	0.01
Apo B/ApoA-I ratio	0.56 $\pm$ 0.02 <sup>b</sup>	0.48 $\pm$ 0.01 <sup>b</sup>	0.49 $\pm$ 0.03	0.52 $\pm$ 0.02	0.04
Fasting plasma glucose, mg/dL	87.46 $\pm$ 9.57 <sup>b</sup>	81.39 $\pm$ 5.78 <sup>b</sup>	80.59 $\pm$ 8.20 <sup>c</sup>	85.21 $\pm$ 8.10 <sup>c</sup>	<0.0001
Insulin, mU/dL	21.38 $\pm$ 9.97 <sup>b</sup>	16.51 $\pm$ 7.75 <sup>b</sup>	17.12 $\pm$ 6.92 <sup>c</sup>	19.65 $\pm$ 8.53 <sup>c</sup>	0.02
HOMA-IR	4.75 $\pm$ 1.46 <sup>b</sup>	3.26 $\pm$ 1.57 <sup>b</sup>	3.27 $\pm$ 1.62 <sup>c</sup>	4.19 $\pm$ 1.05 <sup>c</sup>	0.01
Leptin, $\mu$ g/mL	26.21 $\pm$ 8.64 <sup>b</sup>	23.04 $\pm$ 8.17 <sup>b</sup>	23.95 $\pm$ 6.16	25.93 $\pm$ 7.51	0.04
hs-CRP, mg/L	3.50 $\pm$ 0.35 <sup>b</sup>	1.56 $\pm$ 0.40 <sup>b</sup>	2.06 $\pm$ 0.55 <sup>c</sup>	3.10 $\pm$ 0.72 <sup>c</sup>	0.01
Oxidized LDL, U/L	70.1 $\pm$ 15.4 <sup>b</sup>	65.4 $\pm$ 12.5 <sup>b</sup>	69.8 $\pm$ 16.2	72.2 $\pm$ 18.1	0.01
Malondialdehyde, $\mu$ mol/L	2.71 $\pm$ 0.86 <sup>b</sup>	1.99 $\pm$ 0.35 <sup>b</sup>	1.75 $\pm$ 0.55	1.97 $\pm$ 0.78	0.01

<sup>a</sup>*P* value of variables after receiving zinc supplement versus placebo obtained by paired *t*-test except than for systolic and diastolic blood pressures, zinc, insulin, HOMA-IR and hs-CRP with nonnormal distribution tested by the Wilcoxon signed-rank test.

<sup>b</sup>*P* < 0.05 after versus before receiving zinc.

<sup>c</sup>*P* < 0.05 after versus before receiving placebo.

Abbreviations: SD, Standard deviation; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

TABLE 2. CHARACTERISTICS (MEAN  $\pm$  STANDARD DEVIATION)<sup>a</sup> OF CHILDREN RECEIVING PLACEBO FOLLOWED BY ZINC SUPPLEMENT

	Placebo		Zinc		P
	Before	After	Before	After	
Weight, kg	45.47 $\pm$ 11.00 <sup>c</sup>	47.82 $\pm$ 11.28 <sup>c</sup>	47.51 $\pm$ 11.56 <sup>b</sup>	45.26 $\pm$ 10.30 <sup>b</sup>	0.01
Height, cm	136.61 $\pm$ 7.57	137.00 $\pm$ 7.41	137.60 $\pm$ 7.62	137.96 $\pm$ 7.45	0.07
Body mass index, kg/m <sup>2</sup>	24.71 $\pm$ 3.70 <sup>c</sup>	25.19 $\pm$ 3.64 <sup>c</sup>	25.28 $\pm$ 3.27 <sup>b</sup>	23.75 $\pm$ 3.40 <sup>b</sup>	0.03
Body mass index Z-score, SD	3 $\pm$ 0.38	3 $\pm$ 0.46	3 $\pm$ 0.42	2.5 $\pm$ 0.37	0.01
Waist circumference, cm	82.36 $\pm$ 8.60	82.93 $\pm$ 9.00	82.92 $\pm$ 6.56	81.26 $\pm$ 7.11	0.25
Serum zinc, $\mu$ g/dL	77.68 $\pm$ 28.53	78.09 $\pm$ 21.17	78.08 $\pm$ 22.19 <sup>b</sup>	85.00 $\pm$ 22.41 <sup>b</sup>	0.03
Total cholesterol, mg/dL	180.36 $\pm$ 27.18	179.14 $\pm$ 26.43	178.18 $\pm$ 26.57 <sup>b</sup>	172.37 $\pm$ 25.62 <sup>b</sup>	0.01
LDL-C, mg/dL	108.36 $\pm$ 25.85	109.79 $\pm$ 16.80	109.68 $\pm$ 19.95 <sup>b</sup>	103.15 $\pm$ 21.20 <sup>b</sup>	0.03
HDL-C, mg/dL	45.68 $\pm$ 9.01	43.54 $\pm$ 9.23	42.45 $\pm$ 13.02	42.84 $\pm$ 10.12	0.40
Triglycerides, mg/dL	130.43 $\pm$ 21.95 <sup>c</sup>	134.39 $\pm$ 22.85 <sup>c</sup>	135.45 $\pm$ 20.09 <sup>b</sup>	128.58 $\pm$ 26.01 <sup>b</sup>	0.01
Apolipoprotein A-1, mg/dL	135.00 $\pm$ 14.60	133.65 $\pm$ 17.18	134.82 $\pm$ 14.90 <sup>b</sup>	138.47 $\pm$ 22.39 <sup>b</sup>	0.03
Apolipoprotein B, mg/dL	73.18 $\pm$ 9.17	72.67 $\pm$ 9.81	73.30 $\pm$ 11.84 <sup>b</sup>	69.89 $\pm$ 11.67 <sup>b</sup>	0.01
Apo B/ApoA-I ratio	0.54 $\pm$ 0.02	0.54 $\pm$ 0.02	0.54 $\pm$ 0.03 <sup>b</sup>	0.50 $\pm$ 0.04 <sup>b</sup>	0.04
Fasting plasma glucose, mg/dL	85.46 $\pm$ 9.57	87.39 $\pm$ 5.78	87.21 $\pm$ 8.10 <sup>b</sup>	80.59 $\pm$ 8.20 <sup>b</sup>	0.001
Insulin, mU/dL	21.12 $\pm$ 3.75 <sup>c</sup>	22.75 $\pm$ 3.77 <sup>c</sup>	22.38 $\pm$ 9.97 <sup>b</sup>	19.51 $\pm$ 7.75 <sup>b</sup>	0.03
HOMA-IR	4.12 $\pm$ 1.79 <sup>c</sup>	4.87 $\pm$ 1.54 <sup>c</sup>	4.85 $\pm$ 1.62 <sup>b</sup>	3.91 $\pm$ 1.54 <sup>b</sup>	0.02
Leptin, $\mu$ g/mL	26.21 $\pm$ 8.64	25.04 $\pm$ 8.17	26.05 $\pm$ 8.16 <sup>b</sup>	22.94 $\pm$ 6.51 <sup>b</sup>	0.03
hs-CRP, mg/L	2.39 $\pm$ 0.57	3.39 $\pm$ 0.49	3.15 $\pm$ 0.32	2.43 $\pm$ 0.35	0.01
Oxidized LDL, U/L	70.8 $\pm$ 14.4	70.2 $\pm$ 17.1	70.1 $\pm$ 12.7 <sup>b</sup>	66.5 $\pm$ 11.4 <sup>b</sup>	0.02
Malondialdehyde, $\mu$ mol/L	1.99 $\pm$ 0.65	2.11 $\pm$ 0.86	2.43 $\pm$ 0.05	1.03 $\pm$ 0.04	0.01

<sup>a</sup>P value of variables after receiving zinc supplement versus placebo obtained by paired *t*-test except than for systolic and diastolic blood pressures, zinc, insulin and HOMA-IR, and hs-CRP with no-normal distribution tested by the Wilcoxon signed-rank test.

<sup>b</sup>P < 0.05 after versus before receiving zinc.

<sup>c</sup>P < 0.05 after versus before receiving placebo.

Abbreviations: SD, Standard deviation; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; hs-CRP, high-sensitivity C-reactive protein.

## Discussion

In this trial, which to the best of our knowledge is the first of its kind in the pediatric age group, we found favorable effects of zinc supplementation on anthropometric measures, lipid profile, markers of oxidative stress, inflammation, and insulin resistance. Moreover, the findings of this study corroborate previous data demonstrating the presence of early stages of inflammation and oxidative stress in obesity as early as in early childhood. The results of this trial among nondiabetic obese children are consistent with the beneficial effects of zinc supplementation in adults with established chronic diseases as diabetes.

Metabolic syndrome and oxidative damage are interrelated; this may be partly attributed to lower levels of antioxidant micronutrients, as documented in diabetic patients. Impairments of zinc status have been reported as risk factors in the progression of diabetes. Different mechanisms are suggested for the antioxidant activities of zinc, with potential antioxidant effects in diabetic patients. The effects of zinc supplementation in improvement of insulin sensitivity that can be associated with antioxidant status among diabetic patients might be confirmatory evidence for the role of zinc in these processes. Insulin resistance is associated with increased lipid peroxidation and free radical formation.<sup>22–25</sup>

Simultaneous increases in the production of plasma free radical concentrations and decreases in antioxidant defenses appear to play major roles in oxidative stress in diabetes. Experimental studies have shown that the harmful effects of oxidative stress in diabetes might be associated with the decrease in the synthesis of zinc-containing antioxidant en-

zymes such as superoxide dismutase and glutathione peroxidase.<sup>26</sup> Moreover by considering that hyperglycemia, hyperinsulinemia, and/or insulin resistance increase free radical production, and considering that the common pathway of reduced insulin action by hyperglycemia and insulin resistance might be mediated by oxidative stress,<sup>21–25</sup> it can be suggested that these mechanisms might be involved in the beneficial effects of zinc supplementation in the current trial.

It has been documented that improvement of zinc deficiency in diabetic patients might result in decreased lipid peroxidation and improvements in glucose homeostasis.<sup>25,26</sup> Our comparable findings among young children with metabolic syndrome are confirmatory evidence on the possible modulating effects of zinc on insulin sensitivity and its antioxidant functions.

In a trial among 7 obese men, aged 21–30 years, a 1-month trial of zinc supplementation increased leptin concentrations, but did not modify insulin sensitivity without significant changes in an equal number of controls.<sup>27</sup> However, among our study group of young children, zinc supplementation could clearly improve both leptin concentrations and insulin resistance. This difference might be because our subjects were not only obese, but also had the components of the metabolic syndrome; moreover, our trial comprised a considerably higher number of participants and had a longer duration.

Our findings on the effect of zinc supplementation in decreasing serum LDL-C concentrations are consistent with an 11-week trial of low-dose zinc supplementation among 31 diabetic children aged 5–15 years.<sup>28</sup> The apoB/apoA-I ratio may be a better marker than the lipid profile in predicting cardiovascular risk and may have significant independent

association with insulin resistance in adults<sup>29</sup> and adolescents.<sup>30</sup> A 3-month trial among adults with type 2 diabetes showed that serum levels of ApoA increased in the 17 participants receiving a combination of minerals plus vitamins (Mg + Zn, vitamins C + E), but without significant change in groups receiving either minerals or vitamins.<sup>31</sup>

The current trial was in line with this study in improvement of the apolipoproteins ratio; however, this result was obtained by zinc supplementation without other minerals and vitamins, suggesting that the difference might be because our participants were nondiabetic and were a lower age group, consequently there was a lesser degree of dyslipidemia and early stages of metabolic disorder.

Some aspects of trace element status have been investigated as possible contributory factors to atherosclerosis. Previously, we documented low zinc levels in children of parents with premature coronary heart disease<sup>32</sup>; however, further studies are necessary to explore the role of zinc in early stages of cardiometabolic disorders and atherosclerosis.

### Study limitations and strengths

The follow-up period of this trial was short; some non-significant changes may have become statistically significant with longer follow up. The appropriate dose of zinc supplementation should be assessed in future trials. We could examine the changes in some biomarkers; other markers related to the metabolic syndrome and insulin resistance will be assessed in future trials. The novelty of this trial was including children of very young age with early stages of metabolic, oxidative, and inflammatory disorders. The strengths of the trial were its crossover design to overcome many confounding factors such as genetic background and lifestyle habits among participants, and its triple-masked placebo-controlled design.

### Conclusion

These results are particularly important in light of the deleterious consequences of childhood obesity and early changes in markers of inflammatory and oxidative stress. Although zinc supplementation has not been clinically used for obesity and associated cardiometabolic disorders, given that zinc deficiency is prevalent, and zinc supplementation is being used clinically for other conditions, notably growth disorders, we suggest exploring the direct clinical application of zinc supplementation in childhood obesity in future studies.

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### Author Disclosure Statement

The authors have no conflicts of interest to declare.

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