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Effects of Zinc Supplementation on the Anthropometric Measurements, Leptin, Ghrelin and C-reactive protein in the Obese Adults with Increased Appetite and Baseline Zinc Deficiency: A Randomized Controlled Trial

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ABSTRACT

Background: Appetite is one of the main obesity-controlling factors that can be influenced by hormones, including leptin and ghrelin. This study aimed to determine the effects of zinc supplementation on the serum levels of leptin, ghrelin, C-reactive protein (CRP), and anthropometrical indices in obese individuals with increased appetite and zinc deficiency. **Methods:** This study was conducted among 50 individuals with body mass index $> 30 \text{ kg/m}^2$ in Shiraz, Iran in 2018. The participants were randomly allocated to the intervention that consumed one capsule containing 30 mg/day zinc (ZG) and placebo group (PG) for 3 months. Moreover, all participants were prescribed calorie-restricted diet, 500 kcal/d less than their weight maintenance requirement energy. Anthropometric indices, dietary intake, serum zinc, leptin, ghrelin, and CRP were measured at the baseline and after the intervention. **Results:** The comparison of mean changes in weight (-4.56 ± 2.47 , $P < 0.0001$), body mass index (-1.65 ± 0.85 , $P < 0.0001$), waist circumference (-5.54 ± 4.06 , $P < 0.0001$), hip circumference (-3.19 ± 1.91 , $P < 0.0001$), and serum zinc (15.91 ± 5.24 , $P < 0.0001$) showed a significant difference between groups with greater reduction in the zinc group. However, waist to hip ratio, ghrelin, and CRP showed no significant differences. A significant difference was revealed between groups in terms of the mean leptin concentration changes, in favor of the increase in leptin concentration in ZG ($P = 0.003$). **Conclusion:** Based on the findings, three months of zinc supplementation improved some anthropometric and biochemical measures. Further studies are needed to confirm these results.

Keywords: Obesity; Zinc; Appetite; Anthropometric

Introduction

Obesity is one of the major health challenges in the 21st century, which is defined as high or

abnormal accumulation of fat throughout or specific parts of the body. The prevalence of

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obesity and overweight is constantly increasing and around 1.3 billion adults are obese or overweight globally according to the World Health Organization (WHO) (Arroyo-Johnson and Mincey, 2016, Bray *et al.*, 2016, Reilly *et al.*, 2018, Yanovski, 2018). Obesity and overweight are important determinants of health that lead to negative metabolic changes such as high blood pressure, hyperlipidemia, increased insulin resistance, risk of type 2 diabetes, venous thrombosis, venous embolism, and increased risk of various types of cancers (Bowers *et al.*, 2018, Collaborators, 2017, Staiano *et al.*, 2016). Hence, obesity and overweight impose significant costs on the affected communities and is known to be the fifth cause of death worldwide (Yusefzadeh *et al.*, 2019).

Positive energy balance is the most important cause of obesity in the long run. It is well-known that appetite is one of the most influential factors in energy homeostasis, which is very important in controlling the energy balance (Jastreboff *et al.*, 2019, Romieu *et al.*, 2017). Hormones, such as leptin and ghrelin affect the function of homeostasis centers by altering appetite and causing weight changes (Bray *et al.*, 2016, Cui *et al.*, 2017, Verma and Hussain, 2017).

Ghrelin, a peptide hormone, is appetite stimulant produced by the stomach under the conditions of negative energy balance, such as starvation and weight loss, which can increase the appetite by affecting the appetite centers in the brain (Alamri *et al.*, 2016, Poher *et al.*, 2018, Zigman *et al.*, 2016). Leptin is a polypeptide hormone that is basically secreted from adipocytes (fatty cells). This hormone controls the amount of body fat and controls the body weight through appetite alteration and energy consumption (Pan and Myers Jr, 2018, Stern *et al.*, 2016). Circulating levels of this hormone are lower in individuals with normal weight than obese people. Obese people have high levels of this hormone, but due to the reduced sensitivity of its receptors in the brain, it cannot exert its effects on energy consumption and appetite adjustments, resulting in a condition called leptin resistance in these individuals (Quarta *et al.*,

2016, Simonds and Cowley, 2017, Zhang and Ren, 2016).

Compared to normal weight individuals, obese and overweight people have lower levels of mineral elements, such as zinc, which is known as an effective ingredient in controlling appetite, carbohydrate, lipid metabolism, insulin resistance, and obesity (Fukunaka and Fujitani, 2018, Gu *et al.*, 2018). Circulating levels of zinc in the serum, plasma, and erythrocytes are low in obese individuals (Gawad *et al.*, 2017, Li *et al.*, 2017).

According to the studies, a relationship exists between low plasma levels of zinc and high levels of leptin in obese people. The findings of studies on the relationship between zinc and leptin are contradictory. Some studies did not show any significant changes in the production of leptin with zinc supplementation (Bribiescas, 2003, Olusi *et al.*, 2003), while others reported that zinc supplementation acted as a mediator in the production of leptin and increased its circulating levels (Baltaci *et al.*, 2019, Chen *et al.*, 2000, Hafez *et al.*, 2018).

Weight loss has positive effects on the increase of zinc concentration and zinc supplements can be effective through weight loss mechanisms (Cruz *et al.*, 2017, Gu *et al.*, 2018). Considering the importance of the role of zinc in regulating the level of hormones involved in appetite and macronutrient metabolism, along with the limited studies in the field of zinc supplementation studies on the appetite controlling hormones and anthropometric indices. The present study aimed at investigating the effect of zinc supplementation on leptin, ghrelin, and anthropometric indices in obese or overweight individuals with increased appetite and zinc deficiency.

Materials and Methods

Design and sample size: This study was a double-blinded randomized and placebo-controlled clinical trial conducted in accordance with the declaration of Helsinki and good clinical practice guidelines. The sample size was determined using the result of a previous study (Mantzoros *et al.*, 1998). A sample size of 21 participants in each

group was determined according to the serum leptin difference and with SD of $\sqrt{2}$, type I error of 5%, and power of 90% before the dropouts in each group. Considering the dropouts, the final sample size was 25 participants in each group (A total of 50 participants).

Inclusion and exclusion criteria: Inclusion criteria were the age range of 25-55, body mass index (BMI) $> 30 \text{ kg/m}^2$, serum zinc $< 70 \text{ }\mu\text{g/dl}$, appetite questionnaire score > 20 , absence of vitamin and mineral supplements use over the past 2 months, absence of using any weight loss products or diet, absence of the liver, kidney, thyroid, diabetes, AIDS or other metabolic diseases, lack of using lipid lowering, blood-thinning, and beta-blocker drugs. Exclusion criteria included consumption of less than 90% of the allocated supplements, change in dietary intake or physical activity, and lack of willingness to continue participation.

Sampling and assignment: People who referred to the nutrition ward of Imam Reza Clinic in Shiraz, Iran were examined to select the eligible participants. Initially, after obtaining the written informed consent, blood samples were collected from the participants to determine the serum zinc concentration. As a result, only those with serum zinc concentration of less than $70\mu\text{g/dl}$ were considered as zinc deficient individuals and selected to participate in the study (Mashhadi *et al.*, 2017, Smith *et al.*, 1979). Later, the visual analogue scale questionnaires were filled out to evaluate the appetite score; only those with scores greater than 20 were selected as eligible to continue the study protocol (Zabel *et al.*, 2009). Finally, 50 individuals had the required criteria and were selected to participate in this trial. Participant recruitment was carried out from June to September 2018.

The eligible participants were randomly allocated to either zinc or placebo groups by Blocked randomization (within fixed block size of four) produced by Random Allocation Software (Saghaei, 2004).

Intervention: For three months, the zinc group (ZG) received a capsule containing 30 mg of elemental zinc per day (as zinc gluconate manufactured by Nature Made, Mission Hills, CA), while the placebo group (PG) had received a capsule containing 30 mg indigestible starch. In this study, the 30 mg zinc/day dose was selected because the upper level of zinc intake for adults is 40mg/day (Russell *et al.*, 2001). The placebo capsules were filled in the School of Pharmacy at Shiraz University of Medical Sciences in pre-packed bottles, which were similar in color, size, shape, weight, taste, and odor with the zinc capsules. The bottles were labeled with 2 cods (by a third person, who was not involved in the study and had no contact with participants and investigators) that remained unknown to the researchers until the end of the intervention. Thus, all the investigators and participants were blinded to the treatment assignment.

Participants were asked to take the supplement between meals. Furthermore, similar diets were given to the participants in both groups, including intake of 500 kcal/d less than their estimated energy requirements for weight maintenance based on their usual dietary intakes estimated from 24-hour recalls, which were taken at the beginning of the study.

All participants were asked to maintain their usual dietary habits (regarding 24-hour recalls obtained at the beginning of the study), physical activity (regarding researcher- made questionnaire obtained at the beginning of the study) during the 3 month period of the study. To ensure that the participants would act in compliance with the study protocol and inspect any possible side effects, they were visited by a dietitian on days 30 and 60. Moreover, they were required to return the bottles at every visit, so that the remainder of the capsules could be checked and proper adherence to treatment was defined as consumption of more than 90% of the allocated capsules.

Measurements: All data were collected by trained researchers and all participants were provided with clear instructions. Measurements

were performed at entry and 3 months after the intervention. Demographic information was collected through questionnaires. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg without shoes in light clothes. The participants' BMI was calculated as weight divided by the square of height (kg/m^2). Waist circumference (WC) was measured in cm at the level of the iliac crest at the end of normal expiration. Furthermore, Hip circumference (HC) was measured as the maximum circumference in the hip region and expressed in cm, too. Moreover, waist to hip ratio (WHR) was calculated as WC divided by the HC too.

In the next stage, three-day 24-hour dietary recall (two weekdays and one weekend day) was used for dietary assessment before and after the intervention period. Dietary data from dietary recalls were imported to food processor software (NUT4) and analyzed regarding the Iranian food composition table based on a previous study (Azar and Sarkisian, 1980). Face-to-face interview was conducted to assess the physical activity at the beginning of the study through IPAQ questionnaire and the participants were categorized into three groups of light, moderate, and high physical activity levels.

After 12 hours of fasting, blood samples (10 ml) were collected at the beginning and end of the treatment phase (3 months). Serum samples were separated by centrifugation at 3000 rpm for 10 minutes and stored at -70°C . Upon collection, serum glucose and lipoproteins were determined using standard kits (Pars Azmoon Inc., Tehran, Iran). Serum leptin and ghrelin concentrations were determined by commercially available ELISA kits (Linco Research Inc., USA). The level of C-reactive protein (CRP) was measured using immunoturbidimetric method and serum zinc concentration was estimated by atomic absorption spectrometry (calorimeter) (Chem Tech Analytical, CTA 2000, English).

Ethical considerations: This trial conducted in accordance with the declaration of Helsinki and good clinical practice guidelines. The research protocol, including human participants, was

approved by regional Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1396.125).

Data Analyses: Data were analyzed using SPSS software (version 16:0, Chicago.IL, USA). In all analyses, P-values < 0.05 were considered as statistically significant. Quantitative data were stated as mean \pm standard deviation (SD) and qualitative data were presented as frequency (percentage). Normal distribution of the continuous variables was evaluated by Kolmogorov-Smirnov test. Results obtained from the Kolmogorov-Smirnov test revealed that BMI, leptin, and ghrelin were not normally distributed. Moreover, mean changes (end-of-intervention values subtracted from the baseline values) of all parameters were non-parametrical except for leptin and ghrelin.

Depending on the normal distribution of variables, a paired *t*-test or Wilcoxon signed rank test was used to analyze within group differences before and after the intervention. The difference between the ZG and PG at month 3 was assessed using independent *t*-test (for normally distributed data) and Mann Whitney U test (for non-normally distributed data). In addition, chi-square test was run to examine the differences in qualitative variables between the two groups.

Results

In general, 260 individuals were assessed for eligibility and ultimately 50 individuals were enrolled and randomized, as represented in **Figure 1**. As stated in the flow diagram of the study, two participants were excluded from the zinc group and three from the placebo group. Finally, 23 and 22 individuals were analyzed in the zinc and placebo groups, respectively. Favorably, no adverse effect was reported.

The participants' characteristics are presented in **Table 1**. The findings revealed no significant differences among the study groups regarding gender, education, work status, and physical activity level. Moreover, the results indicated no significant difference in age, weight, and BMI between the study groups at the baseline.

Table 2 shows the calculated dietary intake of 3-day dietary records according to the patients' report. As it is illustrated, no significant differences were revealed at the baseline and three months after the intervention between the two groups, but all dietary variables decreased significantly within the study groups.

Table 3 shows the within group changes in anthropometric and clinical characteristics of the two groups at the baseline and end of the study. Increased leptin was demonstrated only in the zinc group (from 32.43 ng/ml to 39.56 ng/ml, $P = 0.003$). Zinc supplementation significantly decreased weight, BMI, WC, HC, WHR, and CRP, while it significantly increased the serum zinc.

Significant decreases were observed in weight, BMI, WC, and HC of the PG. Interestingly, the results showed a significant increase in the serum zinc in the PG (from 68.95 $\mu\text{g/dl}$ to 70.09 $\mu\text{g/dl}$, $P = 0.018$). Moreover, Ghrelin level stayed unchanged in either group over 3 months of the study period. Comparison of between group changes is displayed in Table 4. The ZG showed greater reduction in weight, BMI, WC, and HC in comparison to the placebo group. Mean changes comparison of WHR, ghrelin, and CRP did not show a significant difference between the two groups. As shown in **Table 4**, the mean differences of zinc and leptin increased significantly in the ZG compared to the PG ($P < 0.001$, $P = 0.003$, respectively).

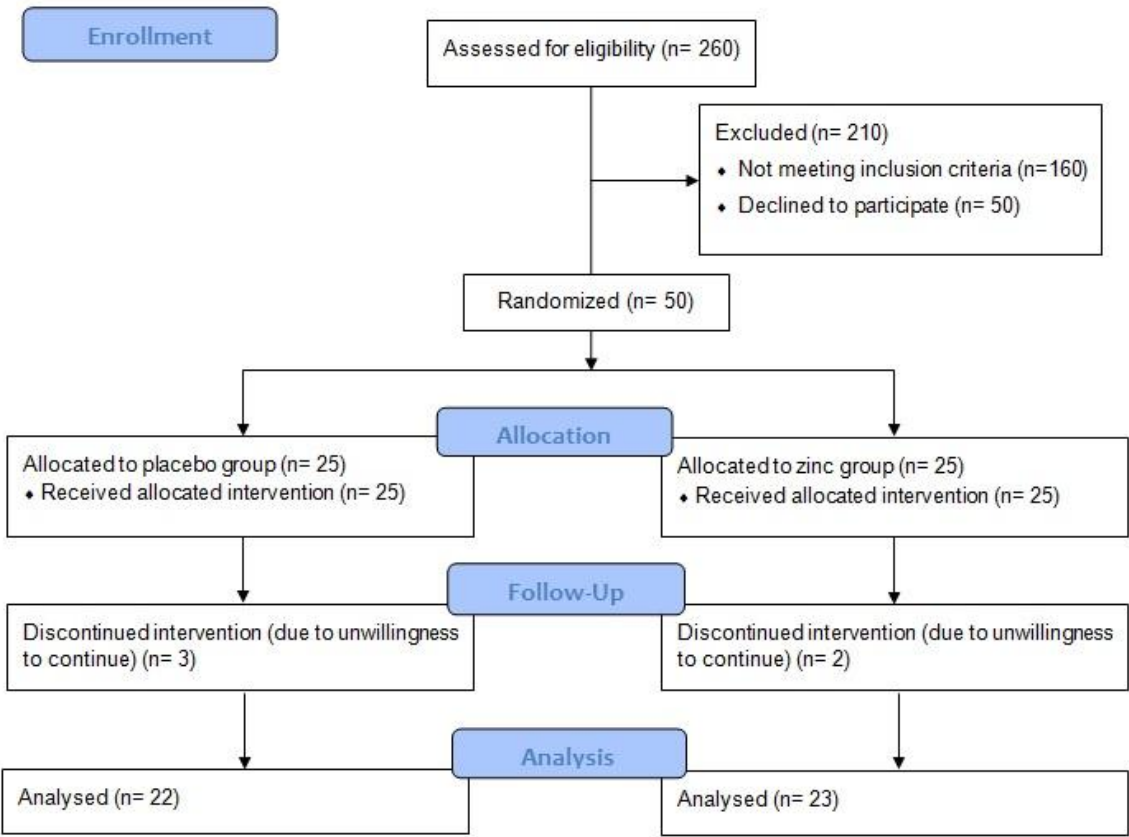


Figure 1. Consort flow diagram of the trial

Table 1. Demographic characteristics of the participants

Variables	Zinc group (N = 23)	Placebo group (N = 22)	P-value
Age (year)	37.31 ± 8.99 ^a	40.00 ± 10.57	0.36 ^d
Weight (kg)	95.64 ± 17.60	93.17 ± 11.58	0.58 ^c
Body mass index (kg/m ²)	34.84 ± 4.69	33.01 ± 1.95	0.26 ^e
Sex			
Female	16 (69.6) ^b	13 (59.1)	0.46 ^c
Male	7 (30.4)	9 (40.9)	
Education status			
Middle school	4 (17.4)	(3) (13.6)	0.96 ^c
Diploma	5 (21.7)	(6) (27.3)	
Bachelor of education	4 (17.4)	(3) (13.6)	
Bachelor of science	5 (21.7)	(5) (22.7)	
Master of science	4 (17.4)	(4) (18.2)	
Doctor of Philosophy	1 (4.3)	(1) (4.5)	
Work status			
Housekeeper	9 (39.1)	(6) (27.3)	0.54 ^c
Clerk	6 (26.1)	(6) (27.3)	
Self-employment	2 (8.7)	(6) (27.3)	
University student	4 (17.4)	(3) (13.6)	
Retired	2 (8.7)	(1) (4.5)	
Physical activity levels			
Light	9 (39.1)	(8) (36.4)	0.98 ^c
Moderate	12 (52.2)	(12) (54.5)	
High	2 (8.7)	(2) (9.1)	

^a: Mean ± SD; ^b: Number (%); ^c: Chi square test; ^d: Independent t-test; ^e: Mann Whitney U test.

Table 2. Mean (±SD) of the participants' dietary intake before and after the intervention

Variables	Zinc group (N = 23)			Placebo group (N = 22)			P-value ^b
	Before	After	P-value ^a	Before	After	P-value ^a	
Energy (kcal/d)	1276 ± 373	864 ± 311	< 0.001	1306 ± 444	961 ± 372	< 0.001	0.15
Carbohydrate (g/d)	209.34 ± 62.05	142.84 ± 43.57	< 0.001	206.07 ± 73.03	156.02 ± 71.35	< 0.001	0.36
Fat (g/d)	27.70 ± 14.29	13.37 ± 6.83	< 0.001	28.50 ± 10.73	23.89 ± 27.40	0.01	0.55
Protein (g/d)	51.14 ± 20.24	34.85 ± 10.66	< 0.001	49.73 ± 21.74	34.89 ± 15.98	0.02	0.37
Zinc (mg/d)	4.78 ± 1.02	3.86 ± 1.37	0.03	4.70 ± 1.04	3.58 ± 1.17	0.04	0.18

^a: Paired *t*-test; ^b: Independent *t*-test

Table 3. Mean (±SD) of anthropometric and clinical characteristics of the participants before and after the intervention

Variables	Zinc group (N = 23)		P-value	Placebo group (N = 22)		P-value
	Before	After		Before	After	
Weight (kg)	95.64 ± 17.60	91.08 ± 16.42	< 0.001 ^a	93.17 ± 11.58	91.85 ± 12.08	< 0.001
BMI (kg/m ²)	34.84 ± 4.96	33.18 ± 4.69	< 0.001 ^b	33.01 ± 1.95	32.52 ± 2.02	0.001 ^b
WC (cm)	117.37 ± 16.34	111.83 ± 15.19	< 0.001 ^a	108.86 ± 19.84	107.09 ± 19.80	0.001 ^a
HC (cm)	119.85 ± 13.54	116.65 ± 13.03	< 0.001 ^a	112.82 ± 14.38	111.36 ± 14.74	0.013 ^a
WHR	0.98 ± 0.10	0.96 ± 0.10	0.002 ^a	0.96 ± 0.11	0.96 ± 0.11	0.533 ^a
Serum zinc (µg/dl)	67.86 ± 8.55	83.78 ± 7.37	< 0.001 ^a	68.95 ± 10.04	70.09 ± 9.23	0.018 ^a
Leptin (ng/ml)	32.43 ± 13.72	39.56 ± 14.65	0.003 ^a	35.00 ± 10.27	32.22 ± 11.02	0.251 ^b

Table 3. Mean (\pm SD) of anthropometric and clinical characteristics of the participants before and after the intervention

Variables	Zinc group (N = 23)		P-value	Placebo group (N = 22)		P-value
	Before	After		Before	After	
Ghrelin (pg/ml)	195.38 \pm 66.82	180.90 \pm 69.04	0.061 ^b	210.09 \pm 53.41	190.73 \pm 64.21	0.061 ^b
CRP (mg/l)	9.88 \pm 2.02	8.69 \pm 1.58	< 0.001 ^a	10.09 \pm 1.84	9.28 \pm 1.51	0.038 ^a

^a: Paired *t*-test; ^b: Wilcoxon signed rank test. BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; CRP: C-reactive protein

Table 4. Mean (\pm SD) difference of Anthropometric and clinical characteristics of the participants

Variables	Zinc group (N = 23)	Placebo group (N = 22)	P-value
Weight (kg)	-4.56 \pm 2.47	-1.32 \pm 1.40	< 0.001 ^a
Body mass index (kg/m ²)	-1.65 \pm 0.85	-0.48 \pm 0.48	< 0.001 ^b
Waist circumference (cm)	-5.54 \pm 4.06	-1.77 \pm 2.11	< 0.001 ^a
Hip circumference (cm)	-3.19 \pm 1.91	-1.45 \pm 2.52	< 0.001 ^a
Waist-to-hip ratio	-0.01 \pm 0.02	-0.003 \pm 0.02	0.059 ^a
Serum zinc (μ g/dl)	15.91 \pm 5.24	1.14 \pm 2.03	< 0.00 ^a
Leptin (ng/ml)	7.13 \pm 12.06	-2.77 \pm 8.77	0.003 ^b
Ghrelin (pg/ml)	-14.47 \pm 60.87	-19.36 \pm 62.72	0.79 ^b
C-reactive protein (mg/l)	-1.18 \pm 0.99	-0.81 \pm 1.71	0.13 ^a

^a: Independent *t*-test; ^b: Mann Whitney U test

Discussion

The results of the present study demonstrated that a combination of calorie deficient diet with 30 mg zinc supplementation per day for 3 months improved the weight, BMI, WC, HC, serum zinc, and leptin significantly, while it led to no significant improvement in WHR, ghrelin, and CRP.

To the best of our knowledge, this is one of the few studies conducted on the effects of zinc supplementation on anthropometric indices and appetite regulatory hormones in obese or overweight adults with increased appetite and baseline zinc deficiency. Appetite regulatory mechanisms are usually disturbed in obese or overweight people. In spite of various treatment options, the lack of proper appetite control is one of the greatest remaining challenges in weight loss programs (do Nascimento Marreiro *et al.*, 2006). Accordingly, finding a solution to improve appetite regulating hormones is highly appreciated in complementing the weight loss programs.

The results of the present study indicated no significant differences in the basic characteristics

of the participants in the study; thus, the probability of affecting the studied outcomes by participants' baseline characteristics was low. The findings showed that the participants had the same food intake at the beginning of the study, but after a calorie-restricted diet, as expected, the intake of calorie and macronutrients in both groups showed a significant decrease. Since comparison of the mean differences between groups was insignificant, outcomes of the study were not affected by dietary intake differences.

According to the present results, serum zinc concentrations increased significantly in the zinc group after the end of the intervention period compared to the control group (mean difference: 15.91 μ g/dl vs. 1.14 μ g/dl, $P < 0.0001$). The results obtained from previous studies confirm this finding. Based on prior studies, the range of 30 to 100 mg/d of zinc sulfate supplementation can improve the serum zinc concentration significantly with no reported adverse effect (Gomez-Garcia *et al.*, 2006, Mantzoros *et al.*, 1998).

In the present study, anthropometrical indices, including weight, BMI, WC, and HC decreased

significantly after supplementation with 30mg zinc for 3 months. Consistent with these findings, Kelishadi *et al.* showed that supplementation with elemental zinc (20 mg/day) on a daily basis for 8 weeks in 60 obese children caused a significant decrease in weight and BMI, but WC changes were not significant (Kelishadi *et al.*, 2010). In another study published by Song *et al.*, 15 days of zinc supplementation in obese male rats resulted in a significant reduction in weight (Song *et al.*, 2009). Possible mechanisms related to the role of zinc in weight loss are altering the metabolism of hypothalamic neurotransmitters, like insulin and insulin sensitizer activity of zinc (do Nascimento Marreiro *et al.*, 2006, Payahoo *et al.*, 2013, Prasad, 2008).

In the present study, serum leptin concentration was significantly increased in the zinc group compared to the control group after the intervention. This finding is in agreement with the results of some previous studies. Gomez *et al.* noted that one-month supplementation with 100 mg zinc (as zinc sulfate) per day in men aged 21 to 30 years (BMI > 27) resulted in a significant increase in serum leptin (Gomez-Garcia *et al.*, 2006). Christos *et al.* also showed that supplementation with 30 and 60 mg zinc in 9 healthy men for 12 weeks increased the leptin levels significantly (Mantzoros *et al.*, 1998).

Chen *et al.* suggested that the possible reason for the effect of zinc on increasing the leptin concentration is to reduce hyperglycemia in obesity or diabetic cases (Chen *et al.*, 2000). It was also demonstrated that zinc supplementation increased the production of interleukin-2 and tumor necrosis factor, including two cytokines which up-regulate the leptin production. Thus, increase in the serum leptin concentration, as a part of the acute-phase response to inflammatory stimuli, such as pro-inflammatory cytokines after zinc supplementation, is the possible mechanism justifying the present result (Gunanti *et al.*, 2016, Kirchgessner *et al.*, 1997, Sarraf *et al.*, 1997). Furthermore, it was stated that zinc affects the leptin-related neurotransmitters and hypothalamic

receptor that have a positive impact on the increase of leptin levels (do Nascimento Marreiro *et al.*, 2006).

The CRP concentration change showed no significant difference between the two study groups. In previous studies, changes in the CRP levels after zinc supplements were attributed to the antioxidant effects of zinc (Bao *et al.*, 2010, Powell, 2000). Antioxidants affect CRP changes by affecting the upstream cytokines, especially IL- β , TNF- α , and IL-6, which are the main drivers of acute phase response. Accordingly, oxidative damage results in inappropriate activation of NF- κ B and leads to increased expression of inflammatory proteins such as CRP (Block *et al.*, 2002, Fischer *et al.*, 2004). It was also suggested that zinc may prevent activation of the above-mentioned pathway (Bao *et al.*, 2010, Powell, 2000). Failure to see a significant change in this study may be due to a low dose or short duration of this study.

Conclusions

Overall, in this randomized double-blind placebo controlled clinical trial, we showed that supplementation with 30 mg zinc per day, along with calorie deficient diet for 3 months, resulted weight, BMI, WC, and HC in obese people. Moreover, serum zinc and leptin concentrations improved, while ghrelin and CRP did not change significantly. This is one of the few studies that evaluated the effect of zinc supplementation on anthropometric indices and appetite regulatory hormone in both genders (obese men and women with increased appetite and baseline zinc deficiency) at the same time. Considering the increasing prevalence of obesity in recent years and the incidence of chronic illnesses caused by it, studies with a longer intervention period are recommended to further clarify and validate the results of the present study.

Authors' contribution

All authors were involved in study design, data management and data analysis and writing the manuscript. They finally verified the final version of the manuscript.

Conflict of Interest

None declared

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