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Dietary Intervention Causes Redistribution of Zinc in Obese Adolescents

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Abstract Obese people tend to have low zinc circulation levels; this is not always related to zinc intake but can reflect the distribution of zinc in relation to the proportion of body fat and factors related to the inflammatory processes that cause obesity. The purpose of this study was to assess zinc distribution in 15 obese adolescent girls before and after a nutritional orientation program. Participants ranged from 14 to 18 years old (postpubescent) and had a body fat percent (BF%) of >35 %. Zinc nutritional status and other zinc-dependent parameters, such as superoxide dismutase (SOD) and insulin levels, were assessed by biochemical analysis of plasma and erythrocytes, salivary sediment, and urine. Samples were collected before and after 4 months of dietary intervention. Dual energy X-ray absorptiometry (DXA) was used to verify BF% both at the beginning and at the end of the study. Food consumption was assessed in ten individual food questionnaires throughout the study; food groups were separated on the questionnaires in the same way as suggested by some authors to develop the Healthy Eating Index (HEI) but with the addition of zinc. After 4 months of nutritional orientation, 78 % of the participants showed a decrease in BF%. Intraerythrocytic zinc increased over the study period, while salivary sediment zinc, SOD, insulin, and Zn urinary24 h/creatinine all decreased ($p<0.05$). There was no difference in zinc intake throughout the study but participants did increase their consumption of fruits, dairy, and meats during the study ($p<0.05$). There were inverse and statistically significant correlations between the increased levels of intraerythrocytic zinc and decreased levels of SOD. There was also a statistically significant correlation between BF% and Zn urinary 24h/creatinine, and SOD. All these parameters were diminished at the end of the study. The dietary intervention for obese adolescent girls is effective with decrease of BF that led to the redistribution of zinc in the body as shown by the changes in erythrocytes, plasma, salivary, urine zinc, as well as the complementary parameters of insulin and SOD. These changes were not affected by zinc intake.

Key words Bioavailability · CuZnSOD · Diet therapy · Obesity · Puberty

Introduction

Many factors, such as genetics, physiology, and metabolism, contribute to the development of obesity. However, the factors that best explain the current rapid increase in the prevalence of obesity are those related to food and lifestyle, such as high energy density diets and low activity levels. These risk factors are especially important in adolescents, whose susceptibility to consumer advertising is a characteristic of the transition to adulthood [1].

Adolescence is a critical period for the development of obesity. During this time, adipocytes are especially prone to develop hyperplasia and hypertrophy [2]. A study conducted by NHANES III (1988–1994) reports that adolescents are also vulnerable to zinc deficiency [3]. Many studies have shown that obesity may exacerbate existing zinc deficiencies

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and that decreased zinc concentrations in the plasma and the erythrocytes are related to either borderline [4] or to very low dietary intake of this element [5].

Studies with animal models [6, 7] and in obese humans [8–10] have demonstrated that subcutaneous adipose tissue synthesizes metallothionein (MT) as well as the stress conditions also increase the levels of MT in the liver [6, 9, 11]. Physiological changes with increasing MT are not yet fully elucidated but some studies indicate that MT sequesters zinc to adipose tissue and the liver, and can lead to losses in the antioxidant functions of MT and the distribution of zinc and copper to tissues [6–11].

Obesity changes the zinc distribution in the human body and favors the production of reactive oxygen species. This can be verified by measuring SOD activity, which, in general, is enhanced in obese animals, in liver [12], and serum [13]. However, human studies suggest that SOD is reduced in the presence of disease; its reduction is usually related to the increase in free radical production [14]. Zinc imbalance in obesity may also affect the membrane signaling related to hormone regulation and favor insulin resistance [15].

The objectives of this study were to evaluate if a nutritional orientation program designed to promote body fat reduction in obese adolescents can be effective in zinc homeostasis and consequently in related biochemical parameters such as insulin and SOD activity.

Materials and Methods

Subjects

This research complied with the ethical guidelines for a study involving human beings according to resolution CNS 196/96 and it was approved by the Ethics Committee of the Pharmaceutical Science School of the University of São Paulo and by the Adolescent Support and Service Center of the Federal University of São Paulo (UNIFESP). The study participants were 15 female adolescents from the city of São Paulo, Brazil who met the following inclusion criteria: 14–18 years old, postpubertal according to the Tanner Classification System, no vitamin or mineral supplementation, no use of any kind of drug, no weight loss for the last 6 months, sedentary lifestyle (i.e., no participation in sports, attendance at a gym, etc.), no orthodontic devices (because zinc can bind to the metal), a body mass index (BMI) of >P85th, and a maximum weight of 125 kg (which is the maximum that can be assessed using our densitometer).

Nutritional Orientation and Dietary Assessment

Every participant attended a clinic twice monthly for a 4-month period. At the clinic, they received information about eating a balanced diet based on three goals: eating every 3 h, eating only one serving (no second helpings), and avoiding snacking between meals. The Brazilian food pyramid was used to provide information about the food groups and guidance as to portion size [16]. Guidelines specifically for adolescent students were also used [17].

During the first month, dietary intake was assessed only once using a questionnaire-based, 24-h food log (R24h). In subsequent months, three R24h were used monthly to assess two weekdays and one weekend day each month. The eating behavior of the participants was assessed using these surveys and food groups were separated based on the groups used to develop the HEI [18].

We evaluated changes in the participants' food selection choices during the study period using selected components of the HEI. The HEI is a standardized measure of diet quality. It divides food intake into dietary components, each of which represents a specific nutritional category and assigns a score based on how closely intake matches nutritional recommendations. In our assessment system, components 1–5 related to the serving recommendations of the food pyramid for the five main food groups: grains (bread, cereal, rice, and pasta), vegetables, fruit, dairy (yogurt and cheese), and meats (including fish, pork, and eggs); component 6 was based on the consumption of beans and oilseeds; components 7 and 8 assessed cholesterol and sodium intake; and component 9 was based on total zinc intake. The oilseeds were grouped with the beans as they are similar in nutritional characteristics. The scores for components 1–6 were determined by comparing the participants' intake with the intake recommended for that food category by the food pyramid [16]. The scores for component 7 were assigned based on the recommendations in the Continuing Survey of Food Intakes by Individuals [19]. The scores for components 8 [20] and 9 [21] were assigned by comparing the participants' intake with the recommended intake for adolescent females of the same age.

To quantify how much each participant consumed of each food component, we categorized the food preparations according to Fisberg and Villar [22] and then calculated calorie food equivalents for each group based on predetermined portion sizes as described in [16]. The Virtual Nutri software [23] was used to quantify the zinc and sodium content of foods; for foods that did not have preloaded values included for these elements, zinc and sodium contents were added using the amounts shown in the tables of McCance and Widdowson [24] and the University Estadual of Campinas [25].

Anthropometric Measures

At the end of each visit to the clinic, the participants were measured using a Welmy brand balance with a capacity of 150 kg and a measurement interval of 100 g. Participants

wore light clothing, were barefoot or wearing socks, and were instructed to stand with their back facing the scales. At the beginning and end of the study, a DXA scanner with a HOLOGIC–4.500 densitometer was used to estimate percent BF%.

Biochemical Parameters

Fifteen milliliters of blood samples were collected in the morning from all participants after a 12-h fast. Blood was distributed into three tubes containing 100 μL sodium citrate at 30 %, 100 μL of EDTA, and without anticoagulants for the analysis of zinc, SOD, and insulin, respectively. Plasma zinc was measured using the methodology described by Rodriguez et al. [26]. Erythrocyte zinc levels was determined using the techniques described by Whithouse et al. [27] and hemoglobin was measured following the standardization of van Assendelft [28].

Urine zinc analysis was performed using flame atomic absorption spectroscopy and expressed as 24-h urine zinc (Zn24h; in microgram per milliliter). For better accuracy, urinary creatinine was also determined using a UV spectrophotometer (HITACHI, Model U1100) at a wavelength of 500 nm with used the kit of reagents (Celm®) and its results were expressed in grams per day [29]. With the two measurements, it is possible to express the ratio between urinary zinc in microgram per creatinine in gram, which is a more accurate expression of zinc excretion than urinary zinc alone.

A 12-h fast is also necessary for saliva collection. Therefore, saliva was collected on the same day as the blood. Participants were told not to brush their teeth with toothpaste that day and upon their arrival at the Adolescent Support and Service Center of the UNIFESP, they were told to rinse their mouths three times with deionized water. They also received a 2×2 piece of parafilm to chew, thus increasing saliva production. A saliva sample of 10–15 mL was collected in a demineralized polypropylene collection vial. The saliva was centrifuged at 14,800×*g* for 10 min and the supernatant was transferred to a demineralized polypropylene vial and stored frozen at −70 °C until analysis [30]. The sediment from the bottom of the vial was dissolved in 800 μL of Milli-Q® water, homogenized using a vortex, transferred to a demineralized polypropylene vial, and stored frozen at −70 ° C. Initially, zinc analysis of the salivary sediment was performed using the gravimetric method. The sediment was then heated to 450 ° C in a laboratory oven to destroy organic matter, ashes were diluted, and zinc was analyzed by the atomic absorption method.

All zinc analyses were conducted using flame atomization in an atomic absorption spectrophotometer (HITACHI model Z-5000) calibrated to a wavelength of 213.9 nm. The reference sample for the analyses was "Second-Generation" Biological Reference Material (freeze-dried human serum for trace element determinations) and standard curve Tritisol ® (MERCK) diluted with deionized water to concentrations of 0.1, 0.2, 0.3, 0.5, and 1.0 μg/mL.

In order to read SOD, it was necessary to protect the erythrocytes stored inside the Eppendorf tubes in aluminum foil frozen at −70 °C. The erythrocytes were then diluted by 300 times with 0.01 M phosphate buffer, pH 7.0, totaling 500 μL of this analytical. Xanthine and oxidase xanthine were used to generate superoxide radicals measured by degree of inhibition and reaction with wavelength of 505 nm in the biochemical analyzer Liasys® AMS with the kit (Ransod; Randox Labs. cat. no. SD 125, Crumlin, UK). This enzyme is expressed in unit per gram of Hb. Hemoglobin (Hb) concentration was verified in the Celm® (E225D) spectrophotometer at a wavelength of 540 nm. Insulin serum concentrations were determined using an Elecsys 1010 immunoassay analyzer with the Roche® insulin kit by the automated electrochemiluminescence method.

Statistical Analysis

Statistical analyses were performed using SPSS. The mean values for plasma zinc, intraerythrocytic zinc, urinary zinc/creatinine, zinc in salivary sediment, body weight, and BF% at the beginning and end of the study were compared using a paired *t* test. For all of these variables, the mean difference between initial and final values met the normality assumption. When the data were not normally distributed, the means were compared using the Mann–Whitney test (insulin only).

Pearson correlation coefficients were used to determine the relationship between biochemical parameters and zinc intake and BF%. The variations in food intake were assessed throughout the study. Adjusted regression was performed to determine the effect of time on the average of these variables while taking into account the correlation between the values obtained for each study participant. These models were adjusted using uniform correlation structures (which assume the same correlation between any pair of observations for each participant, regardless of when the observations were made) or first-order autoregressive correlation (which assumes that the correlation between pairs of observations for each participant decrease when the observations were made further apart in time). Differences are considered statistically significant when $p<0.05$.

Results

Table 1 shows mean ($\bar{x}$) and standard deviation (SD) of body weight, BF%, and biochemical analysis of participant at the beginning and end of the trial. After 4 months of dietary orientation, the mean body weight went from 86.2±12.9 to

85.5±12.5 kg and 53 % of the participants had lost an average of 1.2 kg whereas 40 % had gained an average of 1.3 kg. However, when BF% was analyzed separately, 78 % of the group lost a mean of 4.62±4.2 and 21 % gained 5.1± 5.9 % body fat. With the results obtained using DXA, it was possible to verify that participants 1, 10, and 15, who gained body weight, also gained lean mass and lost fat in grams thus slightly reducing their BF%. Participant number 6, who maintained her body weight throughout the study, gained fat in grams, lost lean mass, and increased her BF%.

Table 1 Results of the weight, %body fat, and biochemical analysis at the beginning and end of the study

Variables	Time			*p*
Weight (kg)	Before	$\bar{x}$	86.2	
		SD	(12.9)	0.74
	After	$\bar{x}$	85.5	
		SD	(12.5)	
Body fat (%)	Before	$\bar{x}$	42. 5	
		SD	(4.2)	0.10
	After	$\bar{x}$	41.5	
		SD	(3.3)	
Plasma zinc (μg/dL)	Before	$\bar{x}$	61.2	
		SD	(8.1)	0.20
	After	$\bar{x}$	63,8	
		SD	(5.5)	
Intraerythocytic zinc (μg/gHb)	Before	$\bar{x}$	35.2	
		SD	(6.4)	**0.002**
	After	$\bar{x}$	41.5	
		SD	(8.7)	
Sediment salivary zinc (ng/ml)	Before	$\bar{x}$	404.4	
		SD	(398.2)	**0.03**
	After	$\bar{x}$	132.8	
		SD	(117.1)	
Intraerythocytic SOD (U/gHb)	Before	$\bar{x}$	1,635.3	
		SD	(419.5)	**0.004**
	After	$\bar{x}$	1,320.6	
		SD	(298.5)	
Serum insulin (μU/ml)	Before	$\bar{x}$	7.6	
		SD	(8.2)	**0.03**
	After	$\bar{x}$	3.6	
		SD	(2.0)	
24-H urine μg Zn/g creatinine	Before	$\bar{x}$	677.36	
		SD	(177.60)	**0.03**
	After	$\bar{x}$	540.64	
		SD	(113.89)	

Statistically significant results are highlighted in bold

In the results of biochemical testing, with reference values for plasma zinc of 70–110 μg/dL [31], at the beginning of the study, 93 % of the participants had plasma zinc levels below the reference range; after the dietary intervention, those values increased but 87 % still did not reach the minimum recommended limit of 70 μg Zn/dL. This increase in plasma zinc was also present in those participants that lost weight and/or BF%, with the exception of one adolescent, who gained BF% and maintained her weight. Plasma zinc levels decreased in 33 % of the participants throughout the study period and these subjects also gained weight and/or BF%.

Adequate levels of intraerythrocytic zinc are 40–44 μg/g of Hb [32]. At the beginning of the study, 67 % of the adolescents had erythrocyte zinc levels lower than the minimum reference value, 20 % of them were within the normal range and 13 % had elevated levels of intraerythrocytic zinc. After dietary intervention, 40 % of participants had erythrocyte zinc levels below the minimum reference value, 20 % were within normal range, and 40 % of the group had levels above the maximum value. Of those participants with elevated erythrocyte zinc levels, 73 % lost body weight and/or BF% over the study period, and the remainder gained weight but lost BF%. When the dietary intervention was not effective (20 %), erythrocyte zinc levels declined from 35.2±8.7 to 31.8±11.0 μg Zn/g of Hb. The mean amount of intraerythrocytic zinc at the end of the trail differed significantly from the mean baseline value.

Values obtained for zinc in the salivary sediment were compared to the mean of values previously reported in two studies of normal female adolescents which was 199± 37.4 ng/mL [30, 33]. At baseline, 27 % of our study participants had salivary sediment zinc levels below the minimum normal value, 27 % were within the normal range, and 46 % were above the maximum normal value. After the dietary intervention, 74 % had salivary sediment zinc levels below normal levels, 13 % remained within the normal range, and only 13 % exceeded the maximum normal value.

SOD reference values are 1,102–1,601 U/g of Hb (RANDOX Laboratories, Crumlin, UK). SOD activity was above the maximum reference value in 40 % of the group. At the end of the study, SOD activity was significantly different from baseline and 60 % of the group had values within the normal range, suggesting that SOD activity changes with dietary intervention.

The reference values for insulin are 6–26 μU/mL [34]. Of the participants, 66 % had insulin levels below the minimum reference value, 27 % were within the reference range, and 7 % had insulin levels above the maximum reference value. After the dietary intervention, 86 % of participants had insulin levels below the minimum reference value, 14 % were within the reference range, and no participants had an insulin level higher than the maximum value. Final insulin levels were significantly different from baseline.

Urine zinc was evaluated in a 24-h urine sample; however, sample volumes affected zinc concentrations and at the end of the study, we determined that assessing zinc levels in relation to urine creatinine was more reliable. Unfortunately, some participants no longer had stored samples at this point and in two participants, we were unable to measure urine creatinine.

At the beginning of the study, 77 % of the participants had creatinine values below the minimum reference level and 23 % were within the normal range for their age group (0.8–1.8 g/day) [35]. At the end of the study, only 17 % of the participants had creatinine levels lower than the minimum reference value, 25 % of them were within the reference range, and 58 % had levels higher than the maximum reference value. In order to obtain the microgram Zn per gram creatinine ratio, it is necessary to divide the amount of zinc in a 24-h urine sample by the amount of creatinine in the same sample; thus, expressing zinc levels in relation to creatinine rather than to urinary volume, which is more variable.

Table 2 shows the consumption of all dietary components throughout the study. The average intake of grains (which includes breads and root vegetables) decreased over the study period, but this decrease was not significant (p=0.988). Vegetable consumption fluctuated between 1 and 2 servings during the entire study period, with no significant differences between any time points (p=0.232). However, fruit consumption was significantly increased during the study (p=0.046), with the average number of servings/day consumed rising from 1.94±2.48 at the beginning of the study to 3.85± 3.77 at the end.

Consumption of milk and dairy products increased an average of 0.189 portions per each R24h showed. Similarly, the lowest amount of meat was consumed at baseline. Consumption of meat increased from 0.80±1.04 servings at baseline to 1.54±1.04 servings at the end of the study.

Consumption of beans and cholesterol varied greatly throughout the study period. At the end of the study, there was a slight increase in consumption, but no significant differences were found (beans, p=0.166; cholesterol, p=0.153). Nuts, which were mainly peanuts, eaten as snacks in school, were also included in this group.

There was a numerical decrease in sodium intake from the initial assessment to the final; however, this difference was not significant. For the quantification of sodium intake in this study, we considered food preparations that included the addition of sodium chloride for salads and other savory prepared foods.

Zinc intake was higher on the weekends in September and October, and decreased at the end of November. The participants reported consumption of barbecue on the days with high zinc intake. Other commonly consumed foods were peanuts (2.130 mg Zn/100 g), red meat (5.0 mg Zn/100 g), and fortified cereal (12.5 mg Zn/100 g) [23]. Zinc intake showed no significant differences throughout the study.

Table 3 shows the correlations between biochemical parameters and zinc intake, intraerythrocytic zinc, and BF%. Zinc intake was not significantly correlated with any biochemical parameters. However, intraerythocytic zinc was correlated with SOD; these are in the same cellular compartment and our results indicate that they both change together (p=0.01). Both urine zinc/creatinine (p=<0.001) and SOD (p=0.049) were correlated with BF%; as weight loss occurred, it resulted in zinc redistribution within the bodies of these obese adolescents.

Discussion

There are differences between changes in total body weight and BF%. Assessment of changes in body condition of obese girls during a growth stage of development, such as adolescence, requires the specific body composition analysis (lean, fat, and bone) that is possible with DXA. In this study, we observed that some participants had their body weights increased and also increased in lean body mass while their amount of fat in grams decreased and their BF% also slightly decreased. Studies that used protocols similar to ours, but included physical activity, also detected subtle decreases in BF% [1]. A 3-month follow-up of a school-based, normocaloric diet intervention for obese adolescents also showed similar results [17].

The dietary guidelines used in this study were developed with two aims: first, the teenagers were asked to decrease their portion size; second, a range of new foods that were not commonly consumed by the participants were gradually introduced to encourage increased consumption of fruit and dairy. Both of these food groups showed significant difference increases in consumption at the end of the study. The decrease in consumption of cereals, breads, and roots by the participants over the study period reflected encouragement to decrease intake of artificial juices, chocolate, candies and cookies.

The meat group also had p values (p<0.05); however, the group received no orientation to increase its consumption, which may reflect the completion of the questionnaires more faithfully during the study. Along with the increase of meats, a tendency of the increase of cholesterol was observed—but it was not statistically significant. The groups of vegetables and beans increased over time and showed a slight reduction of sodium—all without significant difference. The pattern of change in food consumption during the dietary intervention with adolescents is similar to Brazilians adolescents students [17].

Zinc intake in participants was compared to Estimated Average Requirements (EAR) and Recommended Dietary Allowance recommendations [21]. At the beginning of the study, 60 % of the group had zinc intake below the EAR; at

Table 2 Food, cholesterol, sodium, and zinc intake during dietary intervention

Variables	Recommendation		A	S1	S2	SF	O1	O2	OF	N1	N2	NF	*p*
Grains (servings)	5–9 Servings [16]	$\bar{x}$	8.10	6.92	5.87	7.47	6.67	6.56	6.65	6.21	5.73	5.97	0.988
		SD	(4.25)	(2.28)	(1.46)	(3.77)	(2.53)	(1.72)	(1.58)	(3.26)	(2.32)	(2.08)	
Vegetables (servings)	4–5 Servings [16]	$\bar{x}$	1.35	1.54	1.38	1.84	1.19	2.09	1.20	2.07	2.10	1.57	0.232
		SD	(1.51)	(1.64)	(1.85)	(3.32)	(0.80)	(2.34)	(1.53)	(2.36)	(1.81)	(2.10)	
Fruits (servings)	3–5 Servings [16]	$\bar{x}$	1.94	0.84	1.52	1.07	2.04	1.48	1.90	2.03	2.22	3.85	**0.046**
		SD	(2.48)	(1.53)	(1.59)	(1.74)	(2.23)	(1.97)	(2.08)	(3.37)	(2.07)	(3.77)	
Dairy (servings)	3 Servings [16]	$\bar{x}$	1.75	1.92	2.42	2.17	1.81	2.11	1.61	2.21	1.85	1.36	**0.014**
		SD	(1.44)	(1.39)	(1.59)	(2.27)	(1.41)	(1.19)	(0.99)	(1.23)	(1.04)	(1.29)	
Meats (servings)	1–2 Serving [16]	$\bar{x}$	0.80	1.58	1.59	2.52	2.24	1.61	2.30	1.39	2.27	1.54	**0.024**
		SD	(1.04)	(0.94)	(1.58)	(1.95)	(1.41)	(1.36)	(2.12)	(0.96)	(1.48)	(1.04)	
Beans (servings)	1 Serving [16]	$\bar{x}$	0.84	1.61	0.82	0.43	1.08	1.29	1.74	0.60	1.25	1.92	0.166
		SD	(1.20)	(2.46)	(0.72)	(0.73)	(0.97)	(1.34)	(2.93)	(0.75)	(0.77)	(3.47)	
Cholesterol (mg)	214.0±11.0 mg/day [19]	$\bar{x}$	145.10	264.00	195.60	340.10	213.70	262.70	241.30	214.20	305.50	180.90	0.153
		SD	(119.60)	(207.10)	(119.80)	(227.90)	(97.70)	(194.40)	(163.00)	(163.80)	(206.60)	(92.00)	
Sodium (mg)	1,500–2,300 mg/d [20]	$\bar{x}$	2,882.0	2,544.0	2,596.0	2,862.	3,214.0	2,987.0	3,106.0	2,403.0	2,590.0	2,431.0	0.770
		SD	(2,407.0)	(1,093.0)	(1,614.0)	(1,188.0)	(1,672.0)	(1,771.0)	(1,315.0)	(1,195.0)	(1,335.0)	(873.0)	
Zinc (mg)	7.3–9.0 mg/d [21]	$\bar{x}$	7.42	8.92	8.44	12.66	10.77	9.86	11.15	6.92	11.04	6.69	0.808
		SD	(5.45)	(4.38)	(5.90)	(7.39)	(6.10)	(6.00)	(7.32)	(4.39)	(5.66)	(3.83)	

Statistically significant results are highlighted in bold

Month of study: *A* August, *S* September, *O* October, *N* November/*1 and 2 R24h* concerning alternate days of the week, and *F* one weekend day

Table 3 Correlation between biochemical variables and zinc intake, intraerythocytic zinc, and body fat, before and after the dietary intervention

Variables	Before		After	
	r^a	p	r^a	p
Correlation with zinc intake				
Plasma zinc	0.33	0.29	0.54	0.07
Intraerythocytic zinc	0.33	0.29	−0.16	0.62
Salivary sediment zinc	−0.11	0.70	−0.07	0.80
Insulin	0.35	0.21	−0.31	0.26
Urinary zinc/creatinine	−0.09	0.77	−0.30	0.51
Correlation with intraerythocytic zinc				
SOD	0.49	0.07	0.62	**0.01**
Correlation with body fat				
Zinc intake	−0.17	0.55	−0.43	0.11
Plasma zinc	−0.25	0.36	−0.60	0.83
Intraerythocytic zinc	0.31	0.26	0.37	0.18
Salivary sediment zinc	0.06	0.82	−0.16	0.58
Insulin	0.02	0.94	0.41	0.13
Urinary zinc/creatinine	0.08	0.80	0.89	**<0.001**
SOD	0.75	**0.001**	0.52	**0.049**

Statistically significant results are highlighted in bold

[a] Pearson correlation

the end of the study, those values modified and only 20 % of the adolescents had intakes below the EAR. These values for zinc intake are similar to those reported in a study of children and adolescents, also in São Paulo [4]. However, most of the literature reports an inadequate zinc intake in obese subjects [5, 8, 9, 36]. No correlation with zinc intake and biochemical parameters was observed, which supports the theory that mineral redistribution occurs with changes in body composition.

The results of this study show that obese female adolescents are deficient in plasma, erythrocyte, and urine zinc. In contrast, zinc levels in salivary sediment were lower at the end of the study than at baseline. A previous study using obese children and adolescents found increased plasma and intraerythrocytic zinc levels after a weight loss intervention that promoted a protein-rich diet, showing that the increased protein intake increases zinc in both plasma and erythrocytes [37].

Many studies have related BMI to zinc levels; these have shown positive [38] and inverse (high BMI and low zinc levels) [4, 39] correlations. Obese women with a hypocaloric diet tend to lose weight and increase plasma zinc, even when their diet is zinc deficient; the same is not true for males because of their higher demand for zinc [39]. At the beginning of this study, we observed zinc levels lower than those considered adequate both in plasma and in the erythrocytes; however, after the dietary intervention, there was an increase in plasma zinc levels in 67 % of the adolescents ($p>0.05$) and in intraerythrocytic zinc levels in 80 % of participants ($p<0.05$).

Different tissues (lean or fat) express different types of zinc transporter proteins and even intra-abdominal and subcutaneous fat can have different zinc transporter protein expression. Adipose tissue contributes largely to the storage of zinc [40]. In addition, inflammatory states, such as obesity, increase the synthesis of metallothionein, which in turn sequesters zinc, consequently reducing its levels in the blood. This has been demonstrated in both animal models [6, 7] and in obese humans [8, 9, 41]. Based on this, we anticipated that blood zinc levels would increase with a decrease in body fat in obese individuals, thus creating a mechanism for mineral homeostasis.

Salivary zinc may be more closely related to actual zinc intake, especially in vegetarian women [42] with low zinc intake (3.2 mg/day) [30]. There is also redistribution of zinc with age; Bailes et al. [43] found that in healthy subjects of varying ages, salivary zinc increased with increasing age. Higher zinc levels were found in the salivary supernatant of elderly people with higher body weights than in younger patients with less weight. However, no difference was observed in zinc levels in other components of saliva.

Studies with eutrophic adolescents found zinc levels of 222±161 ng Zn/mL in saliva when adolescents consumed a diet containing 14.7 mg/day of zinc and 203±72 ng Zn/mL of zinc in saliva when the dietary intake was 11.5 mg/day of zinc for a period of 15 days [33]. In this study, zinc levels in the salivary sediment were higher at baseline than at the end of the study and were not correlated with zinc intake, which was higher at the end of the study than at baseline.

However, the 67 % of the participants who decreased in salivary sediment zinc over the study period paralleled the 53 % that also lost weight and BF%. After the dietary intervention, the participants had salivary zinc levels that are more similar to those reported in the literature; this reflected both the reduction in zinc concentrations in the salivary sediment and the increase in zinc intake. Another interesting fact is that the proportion of zinc in saliva is not related to its levels in plasma and hair, suggesting that caution should be used when evaluating this parameter.

Guthrie and Picciano [32] mention that urinary zinc excretion is reduced as a homeostatic response to maintain zinc levels in plasma when there is a dietary zinc deficiency. However, urinary zinc concentrations also respond to changes in muscle catabolic processes and might be increased in some disease states [44] including obesity and during bariatric surgery [5].

The urinary zinc levels found in these adolescents reflect the low 24-h urine volume at the beginning of the study. However, when those values were expressed as microgram Zn per gram of creatinine, it was possible to observe a higher loss of zinc at the beginning of the study than at its end. On the other hand, in obese adolescents undergoing weight loss, an increase in creatinine is expected since it is more related to lean mass than to body weight [29].

Studies have shown the relationship between insulin and hyperphagia in obese individuals. The presence of insulin in the cerebrospinal fluid has led to the hypothesis that it regulates food intake and body weight because satiety is proportional to adiposity [45]. Fifty-three percent of the adolescents demonstrated weight and/or BF% loss and 20 % presented weight and/or BF% gain suggesting a trend between weight and BF% loss and a reduction in insulin levels.

Roemmich et al. [46] reported that the higher the visceral fat, the higher the insulin levels. This confirmed the results reported by Lemieux et al. [47] who stated that insulin homeostasis might be different between individuals, depending on how long the person has obesity and on body fat distribution. We verified that a small decrease in body fat in obese adolescent girls during the dietary intervention was associated with increased zinc levels in the bloodstream and decreased insulin production. This would result in improved glucose utilization by the peripheral tissues as well as increased activity of the insulin-independent glucose transporters (GLUT 1 and 2) [46].

Infectious processes cause an excess of reactive oxygen species, which leads to zinc deficiency and decreased insulin production. This can be explained by the formation of the Zn–MT complex and other antioxidants that are formed from essential nutrients such as zinc, resulting in mineral deficiency and an increased production of body-protecting enzymes [48].

However, the assessment of these process antioxidants enzymes is extremely specific because the inflammatory process found in obesity and SOD activity is very sensitive for each type of individual taking into consideration regarding sexual maturation, gender, age, and time that the individual had excess of BF [49]. The assessment of SOD becomes complex because of the cascading effect that has to exert its antioxidant role; first of reactive oxygen substances that removes then converts superoxide anions to hydrogen peroxide to (H_2O_2) and H_2O_2 can be rapidly degraded by catalase and GPx to H_2O [50, 51].

Studies with human condition that are overweight in general show decreased SOD, which can be understood as a physiological mechanism of the importance of the enzyme in the model of compensatory adjustment to oxidative stress. Some studies have revealed that the SOD in serum [52, 53] and erythrocytes [12, 54, 55] of obese individuals is at lower levels when compared to the control groups.

In our study, we found that dietary intervention aided in the distribution of zinc in the body because after 4 months, there was a redistribution of zinc to the plasma and erythrocytes showing a significant correlation between increased erythrocyte SOD with decreased well as the BF.

This biological change makes us understand that the decrease in fat distributes the zinc into the bloodstream and that decreased body fat causes a possible generation of reactive oxygen species in turn, thereby causing the body to have a lesser need of antioxidant enzymes such as SOD.

Another fact that can also be questioned is the absence of enzymatic activity of substances with antioxidant functions—such as MT—which in this case, also remained free because there were no sequesters and there are more zinc in adipose tissue and liver.

The increase in oxidative stress can sometimes be extreme, such as when the reduction of the release of the reactive oxygen species by the zinc-dependent enzymes leads to an increase in their levels, and produces zinc deficiency as a secondary effect [56].

In conclusion, the improvement in the standard of food to obese adolescents girls causes a slight diminution of BF, altering the levels of oxidative stress, observed by decreased SOD and increased zinc in erythrocytes. The changes in salivary sediment zinc levels may be a promising parameter for obesity research since the changes in the amount of zinc in saliva were opposite to those of other zinc-related biochemical parameters.

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