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### Effect of Zinc Supplementation on GH, IGF1, IGFBP3, OCN, and ALP in Non-Zinc-Deficient Children

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## Original Research

# Effect of Zinc Supplementation on GH, IGF1, IGFBP3, OCN, and ALP in Non-Zinc-Deficient Children

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**Key words:** venous zinc administration, oral zinc supplementation, GH-IGF1 system, growth, children

**Objective:** Because most publications on growth and development deal with children with zinc deficiency, we decided to study the effects of this micronutrient on the secretion of growth hormone (GH), insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3), osteocalcin (OCN), and alkaline phosphatase (ALP) in healthy and eutrophic children. This study is original because the methodology was unique.

**Methods:** Forty schoolchildren participated in the study, 17 females and 23 males, aged 8 and 9 years. The study was carried out during a 3-month period. It was characterized as a triple-blind randomized controlled trial. The children were divided in a control group (20 schoolchildren using 10% sorbitol) and experimental group (20 schoolchildren using zinc). All were submitted to oral zinc supplementation (10 mg Zn/day) and venous zinc administration (0.06537 mg Zn/kg of body weight). Blood samples were collected at 0, 60, 120, 180, and 210 min. All schoolchildren were also submitted to anthropometric, clinical, and dietetic assessments as well as biochemistry analyses.

**Results:** Oral zinc supplementation in the experimental group (1) stimulated an increase in the consumption of protein and fat ( $p = 0.0007$ ,  $p < 0.0001$ ,  $p < 0.0001$ , respectively), (2) increased basal serum zinc ( $p < 0.0001$ ), (3) increased plasma ALP ( $p = 0.0270$ ), and (4) showed a positive correlation for IGF1, IGFBP3, and OCN, comparing before and after oral zinc supplementation ( $p = 0.0011$ ,  $p < 0.0001$ ,  $p < 0.0446$ , respectively). During zinc administration, plasma IGF1 and IGFBP3 increased significantly in the experimental group ( $p = 0.0468$ ,  $p < 0.0001$ , respectively). Plasma GH increased in the experimental group but without statistical difference comparing before and after oral zinc supplementation.

**Conclusions:** Zinc supplementation stimulated an increase in the consumption of some macronutrients and basal serum zinc and improved plasma alkaline phosphatase levels. Zinc administration increased hormones of the GH-IGF1 system.

## INTRODUCTION

Since the firsts publications of Prasad et al. in the 1960s [1], zinc assumed a role of importance in human and animal nutrition [2].

The micronutrient zinc plays a role in the mechanisms of secretion and peripheral action of the hormones involved in the growth of both human and animal models [3, 4]. The effect of oral zinc supplementation on serum zinc status changes depending on the duration of the intervention, chemical formula, dose of supplementation, and nutritional situation [5]. Moreover, oral zinc supplementation was more effective than an adequate animal protein diet for growth in children [6].

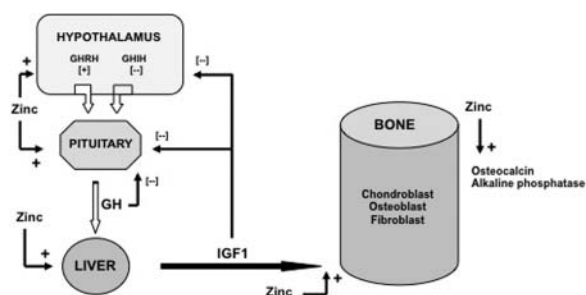
The principal system that regulates growth is the hypothalamus–pituitary–liver–bone. Most studies show the positive effect of zinc on the growth of children with zinc deficiency, although the mechanisms are not fully understood [7].

Regarding growth hormone (GH), it should be noted that zinc stimulates or induces (Fig. 1) hypothalamic neuropeptides [8], physiological GH secretion [7,9], sensitivity of endogenous GH [10], binding of GH to its receptors [11], bioactivity of GH [12], dimerization of GH [13], expression of GH receptor and insulin-like growth factor 1 (IGF1) genes in the liver [14], and GH postreceptors [15].

The hormonal concentrations of insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding protein 3

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## Effect of Zinc on GH-IGF1 System in Children



**Fig. 1** Action of zinc on longitudinal bone growth. It stimulates the action of growth hormone-releasing hormone (GHRH), which in turn acts on pituitary somatotrophic cells, which synthesize and secrete growth hormone (GH). This hormone acts on liver receptors that express IGF1 synthesis. Its biological action occurs mainly in the epiphyseal chondrocytes, responsible for longitudinal bone growth. Thus, zinc acts at all levels of the mechanisms responsible for bone growth. *Note.* GHIH = somatostatin.

(IGFBP3) also increase with age in children, particularly in the pubertal catchup [16]. These hormones are influenced by zinc because zinc deficiency decreases circulating IGF1 and IGFBP3 concentrations [17], which are increased after oral zinc supplementation, including in non-zinc-deficient children [3,17]. Regarding IGF1, zinc appears to be essential for IGF1 induction of cell proliferation [18], activation of IGF1 receptor tyrosine kinase [19], and hepatic IGF1 gene expression [20].

Osteocalcin (OCN) is a noncollagenous, vitamin K-dependent protein [21], and its serum concentrations are higher throughout childhood, markedly at puberty in girls and boys [22]. Moreover, OCN mRNA levels were positively correlated to zinc exposure in a murine osteoblast-like cell line [23]. Oral zinc supplementation increased serum OCN in short children with marginal zinc deficiency [24] and in MC3T3-E1, a murine osteoblastic cell line [23]. Taken together, zinc interacts at multiple steps of synthesis, secretion, and action of hormones, essential for linear growth.

Alkaline phosphatase (ALP) is a byproduct of osteoblast activity, associated with bone formation, and tartrate-resistant acid phosphatase (TRAP) is an enzyme that is expressed by bone-resorbing osteoclasts. The synthesis of ALP is impaired in children with zinc deficiency [3].

Thus, we aimed to evaluate the effect of oral zinc supplementation and venous zinc administration on GH, IGF1, IGFBP3, OCN, and ALP in healthy and eutrophic children using a physiological dose of zinc. There are very few studies in the literature conducted under these conditions, and our study used an innovative methodology.

## MATERIAL AND METHODS

### Subjects

Forty prepubertal schoolchildren participated in the study, including 17 females and 23 males, aged 8 and 9 years, from 4

municipal schools in the northeastern city of Natal, Brazil. The students were authorized by their parents or guardians to take part in the study, and the study was approved by the Onofre Lopes University Hospital Research Ethics Committee at the Federal University of Rio Grande do Norte (UFRN), Brazil (number 323/09).

### Selection Criteria

Inclusion criteria were healthy schoolchildren, as assessed by laboratory tests and clinically by an endocrinologist and anthropometrically by nutritionists. Moreover, all schoolchildren should be in the Tanner stage I for genital, breast, and pubic hair growth [25] and with body weight, height, and body mass index (BMI) within the normal reference range for age. Exclusion criteria included schoolchildren who were underweight, overweight, or obese, and those with serum zinc below  $0.7 \mu\text{g/mL}$ ; Tanner stage II; acute, chronic, infectious, or inflammatory diseases; or nutritional disorder, and children who had undergone surgery or were using vitamin and mineral supplements.

### Experimental Design

The study was carried out during a 3-month period for a good action of zinc on biological mechanisms. It was characterized as a triple-blind randomized controlled trial, formed by a process of nonprobability sampling (convenience sample), in which participants were divided into control and experimental groups. The pairing was done randomly. The control group consisted of 20 schoolchildren (9 female and 11 male, using placebo). The experimental group consisted of 20 schoolchildren (8 female and 12 male, using oral zinc). Serum zinc, dietary zinc intake, GH, IGF1, IGFBP3, OCN, alkaline and acid phosphatase, total proteins, and hemoglobin were observed before and after oral zinc supplementation.

### Anthropometric Assessment

We measured the body weight (in kilograms) and height (in centimeters) using an electronic balance (BK50F, Balmak, São Paulo, SP, Brazil) and a stadiometer (Stadiometer Professional Sanny, American Medical do Brasil, São Paulo, SP, Brazil), respectively. The analysis of nutritional status was also based on BMI for age using the new growth curves published by the World Health Organization [26], which rebuilt the growth reference recommended by the National Center for Health Statistics for children from 5 to 19 years of age. We calculated this parameter using an online program [27]. Nutritionists performed anthropometric assessments.

### Dietetic Assessment

Food intake evaluation was performed with a prospective 3-day food record on 2 weekdays and 1 on the weekend. Mothers

were instructed to record each of the following about the child's food intake: energy, macronutrient, fiber, calcium, iron, and zinc intake was calculated with NutWin software version 1.5 [28]. Foods not included in the program were inserted based on food chemical composition tables [29]. Nutritionists performed nutritional assessments.

### Oral Zinc Supplementation

The control group received an oral placebo as sorbitol 10%. The experimental group received 10 drops of zinc solution (10 mg Zn/day), as zinc sulfate heptahydrate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , Merck, Darmstadt, Germany). Both were added to milk or juice every morning at breakfast. Zinc supplementation of 10 mg/day did not exceed the values of the tolerable upper intake level in the age groups mentioned. Syrups were prepared at the Pharmacotechnical Laboratory of the Department of Pharmacy, UFRN. Zinc ingestion was controlled every 2 weeks by the same observers who performed previous measurements.

### Venous Zinc Administration

A bolus of 0.06537 mg Zn/kg of body weight ( $1 \mu\text{mol ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) was injected intravenously in all schoolchildren in the experimental group at time 0 min over the course of 1 min. Each 5 mL ampoule contained  $40 \mu\text{mol ZnSO}_4 \cdot 7\text{H}_2\text{O}$ . These ampoules were prepared at the InjectCenter—Handling of Injectables (Ribeirão Preto, São Paulo, Brazil). An antecubital vein of each forearm was catheterized and maintained with metal-free saline. Venipuncture was performed using plastic metal-free syringes without a tourniquet. Zinc was injected at 8:00 AM. Blood samples were collected at 0 (before venous zinc administration), 60, 120, 180, and 210 min from the contralateral arm. A medical doctor and his staff monitored all schoolchildren throughout the test.

### Zinc Analyses

Zinc samples were stored for 2 hours at  $37^\circ\text{C}$  in a stainless steel incubator (502, Fanem, São Paulo, Brazil) to allow clotting and serum separation. Hemolytic samples were discarded, because erythrocytes are rich in zinc [30]. Serum samples were diluted to 1:4 with ultrapure water (Milli-Q Plus, Millipore, Billerica, MA) and then stored at  $-80^\circ\text{C}$  (Ultralow Freezer, Nuaire, Plymouth, MN) until analysis.

Serum zinc was measured using an atomic absorption spectrophotometer (SpectrAA-240FS, Varian, Victoria, Australia). Calibrations and measurements were carried out in accordance with the manufacturer's instructions. The sensitivity was  $0.01 \mu\text{g/mL}$ , the intra-assay coefficient of variation was 2.2%, and the reference values were  $0.7\text{--}1.2 \mu\text{g/mL}$ . We used a standard control of serum prepared by the laboratory itself and handling of zinc samples was performed according to international standards [30].

### Hormones Analyses

GH, IGF1, IGFBP3, and OCN were measured by chemiluminescence (IMMULITE 1000 Immunoassay System, Siemens, Washington, DC). GH sensitivity was  $0.01 \text{ ng/mL}$ , the intra-assay coefficient of variation was 7.8%, and the reference values were up to  $10 \text{ ng/mL}$ . IGF1 sensitivity was  $20 \text{ ng/mL}$ , the intra-assay coefficient of variation was 10.5%, and the reference values were  $60\text{--}390 \text{ ng/mL}$ . IGFBP3 sensitivity was  $0.1 \mu\text{g/mL}$ , the intra-assay coefficient of variation was 11.8%, and the reference values were  $1.6\text{--}7.1 \mu\text{g/mL}$ . OCN sensitivity was  $0.6 \text{ ng/mL}$ , the intra-assay coefficient of variation was 11.6%, and the reference values were not established.

### Hematological and Biochemistry Analyses

Hematological analyses were measured using the standard clinical laboratory method (Horiba ABX Diagnostics, Micros 60, Montpellier, France). Total proteins, ALP, and TRAP were measured by a colorimetric method in a biochemical analyzer (Dade Behring Dimension AR, Deerfield, IL).

### Statistical Analyses

Statistical analyses include the D'Agostino & Pearson omnibus normality test to analyze the normality of all study data. Paired and unpaired Student's *t* tests were run to compare the data obtained on the control and experimental groups or between both. The Wilcoxon matched-pairs signed rank test was used to complement paired nonparametric tests and the Mann-Whitney test was used to complement unpaired nonparametric tests. BMI, total energy intake, zinc, and hormones were analyzed in terms of the area under the curve (AUC). Correlation was used to measure 2 variables in each subject with Pearson correlation coefficients and Spearman's for nonparametric correlation. The selected level of significance was  $p = 0.05$ . Statistical tests were performed using GraphPad Prism 6.0 software (San Diego, CA).

## RESULTS

### Subjects

Forty prepubertal schoolchildren presented chronological ages of  $8.6 \pm 0.5$  years (control group) and  $8.9 \pm 0.5$  years (experimental group). All were in Tanner stage I.

### Anthropometric Assessment

Weight was significantly different at the end of the study for control and experimental groups. Height increased significantly in both control and experimental groups. All schoolchildren were eutrophic during the 3-month study and BMI for age was significantly different at the end of the study only for

**Table 1.** Weight, Height, and Body Mass Index Values. All Values Were Obtained before and after Oral Zinc Administration in the Control and Experimental Groups of Eutrophic and Healthy Children<sup>a</sup>

	Control			Experimental		
	Before	After	<i>p</i> Value*	Before	After	<i>p</i> Value*
Weight (kg)	27.69 ± 0.99	28.70 ± 1.14	0.0005	27.62 ± 1.02	28.59 ± 1.05	<0.0001
Height (cm)	130.70 ± 1.48	132.00 ± 1.50	<0.0001	131.40 ± 1.42	132.90 ± 1.45	<0.0001
Body mass index (kg/m <sup>2</sup> )	16.13 ± 0.39	16.39 ± 0.45	0.0592	15.81 ± 0.30	16.11 ± 0.34	0.0489

C-B = control before, C-A = control after, E-B = experimental before, E-A = experimental after.

<sup>a</sup>Values are expressed as mean ± SEM.

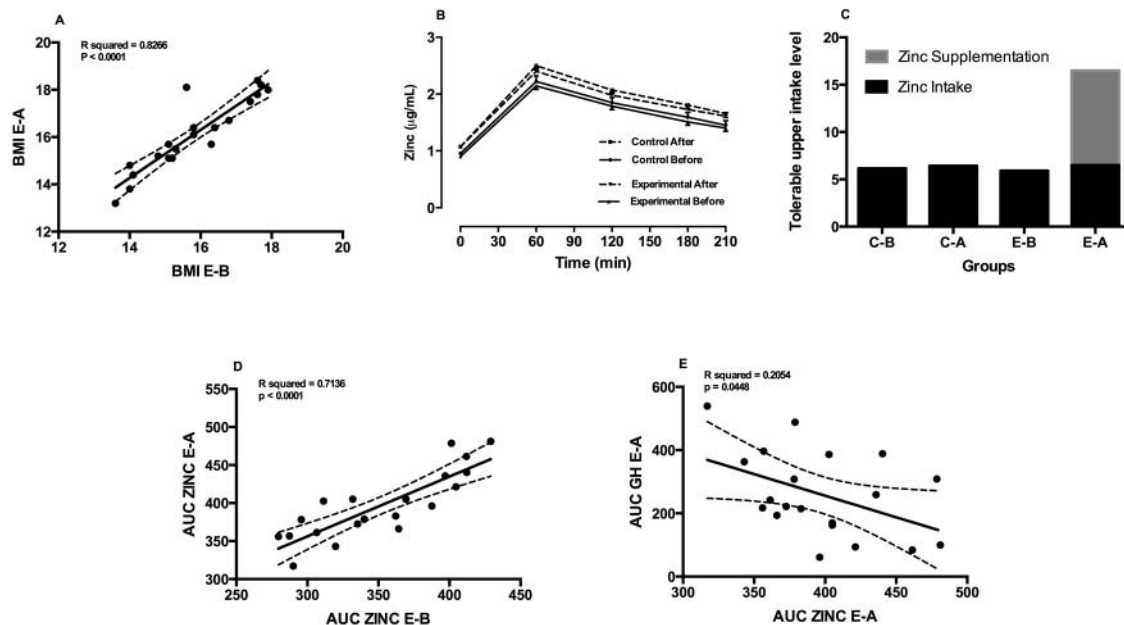
\**p* Values for paired Student's *t* tests in the control and experimental groups. The remaining comparisons were conducted using paired and unpaired tests as follows: (a) Weight: C-A × E-B = 0.4848, C-A × E-A = 0.9437, C-B × E-B = 0.9651, C-B × E-A = 0.5342; (b) Height: C-A × E-B = 0.7683, C-A × E-A = 0.6899, C-B × E-B = 0.7403, C-B × E-A = 0.3075; (c) BMI: C-A × E-B = 0.2906, C-A × E-A = 0.6181, C-B × E-B = 0.5234, C-B × E-A = 0.9695. *p* < 0.05 was considered significant.

experimental group (Table 1). The AUC of BMI showed a strong positive correlation only in the experimental group after oral zinc supplementation (Fig. 2A).

### Dietetic Assessment

All values of macro- and micronutrient intake were not significant in the control group. The experimental group only significantly increased the values of energy, protein, and total fat after oral zinc supplementation; the values of fiber and calcium were decreased in the experimental group. Iron and zinc intake was in the normal range for the control and experimental groups

(Table 2) [31–34]. The mean apparent adequacy for calcium was −1.2 and −1.3 (inadequate for the control group before and after placebo, respectively) and −1.5 and −1.3 (inadequate for the experimental group before and after zinc supplementation, respectively). The mean apparent adequacy for iron was 1.1 and 1.2 (adequate for the control group before and after placebo, respectively) and 1.2 and 1.3 (adequate for the experimental group before and after zinc supplementation, respectively). Finally, the mean apparent adequacy for zinc was 0.4 and 0.4 (adequate for the control group, before and after placebo, respectively) and 0.3 and 0.3 (adequate for the experimental group, before and after zinc supplementation, respectively).



**Fig. 2** (A) Correlation between BMI E-B and BMI E-A before and after oral zinc supplementation in the experimental group; (B) serum zinc profiles during venous zinc administration in control and experimental groups; (C) zinc intake in the control group and zinc intake plus zinc supplementation (10 mg Zn/day) in the experimental group; (D) a positive correlation was observed in the experimental group before and after zinc supplementation; (E) correlation between AUC ZINC E-A and AUC GH E-A after oral zinc supplementation in the experimental group. Notes. E-B = experimental before; E-A = experimental after. Values are expressed as the mean ± SEM, R squared and *p*. A *p* value of < 0.05 was considered significant.

**Table 2.** Energy and Nutrient Intake before and after Oral Zinc Supplementation in Control and Experimental Groups Compared to Recommendations by Age and Sex<sup>a</sup>

	Before Supplementation	<i>p</i> Value*	After Supplementation	<i>p</i> Value*	Reference Value
Energy (kcal)					
Control group	1563 ± 42.45	0.1463	1573 ± 43.77	0.0007	6–9 years (boys): 1573–1978 kcal/day [31]
Experimental group	1647 ± 37.80		1814 ± 48.64		6–9 years (girls): 1428–1854 kcal/day [31]
Protein (g)					
Control group	40.22 ± 0.47	0.6993	45.55 ± 0.51	<0.0001	4–8 years (both sexes): 0.76 g/kg/day [32]
Experimental group	41.08 ± 0.69		46.89 ± 0.665		9–13 years (both sexes): 0.76 g/kg/day [32]
Fat (g)					
Control group	35.91 ± 0.49	0.9891	36.10 ± 0.49	<0.0001	ND [32]
Experimental group	35.92 ± 0.38		39.33 ± 0.56		
Carbohydrate (g)					
Control group	182.70 ± 2.34	0.2288	183.90 ± 2.31	0.0758	100 g/day [32]
Experimental group	182.60 ± 3.96		185.10 ± 4.04		
Fiber (g)					
Control group	10.56 ± 0.25	0.4741	11.04 ± 0.24	0.0893	4–8 years (both sexes): 25 g/day [32]
Experimental group	10.30 ± 0.26		11.67 ± 0.22		9–13 years (girls): 26 g/day [32]
Calcium (mg)					9–13 years (boys): 31 g/day [32]
Control group	647.40 ± 25.20	0.1192	636.90 ± 21.46	0.7911	4–8 years (both sexes): 800 mg/day [33]
Experimental group	578.50 ± 17.13		630.40 ± 11.71		9–13 years (both sexes): 1100 mg/day [33]
Iron (mg)					4–8 years (both sexes): 4.1 mg/day [34]
Control group	8.79 ± 0.17	0.4682	9.00 ± 0.16	0.1085	9–13 years (boys): 5.9 mg/day [34]
Experimental group	8.65 ± 0.09		9.25 ± 0.08		9–13 years (girls): 5.7 mg/day [34]
Zinc (mg)					
Control group	6.15 ± 0.11	0.1146	6.43 ± 0.10	0.7086	4–8 years (both sexes): 4 mg/day [34]
Experimental group	5.91 ± 0.10		6.48 ± 0.10		9–13 years (both sexes): 7 mg/day [34]

ND = not determined.

<sup>a</sup>Values are expressed as the mean ± SEM.\**p* < 0.05 (unpaired Student's *t* tests).

### Zinc Analysis before and after Oral Zinc Supplementation

Basal serum zinc concentrations increased in both study groups. However, it was statistically significant in the experimental group, *p* < 0.0001 (Fig. 2B). These concentrations remained in the normal reference range and no side effects were observed with a dose of 10 mg Zn/day. The value of tolerable upper intake levels are shown in Fig. 2C.

### Zinc Analysis before and after Venous Zinc Administration

Serum zinc curves in both groups studied increased significantly during venous zinc administration (Fig. 2B). The values of the AUC of zinc increased in the control group after oral placebo, *p* = 0.0004, and more significantly in the experimental group after oral zinc supplementation, *p* < 0.0001. There was a positive correlation between the AUC of zinc in the experimental group (Fig. 2D) and a negative correlation between the AUC of zinc and the AUC of GH in this same group, *p* = 0.0448 (Fig. 2E). However, no correlation was present between the AUC of zinc and the AUC of IGF1, IGFBP3, and OCN (data not shown). No adverse effects were observed during zinc injection.

### GH Analysis

GH increased in the control and experimental groups after venous zinc administration (Fig. 3A). However, the analysis of

the AUC showed no significant changes in plasma GH concentrations between both groups studied (data not shown). There was no correlation between the AUC of GH in the experimental group (Fig. 3B). Moreover, no of correlation was observed between the AUC of GH and the AUC of IGF1, IGFBP3, and OCN in the experimental group (data not shown). All concentrations of this hormone were within the normal range.

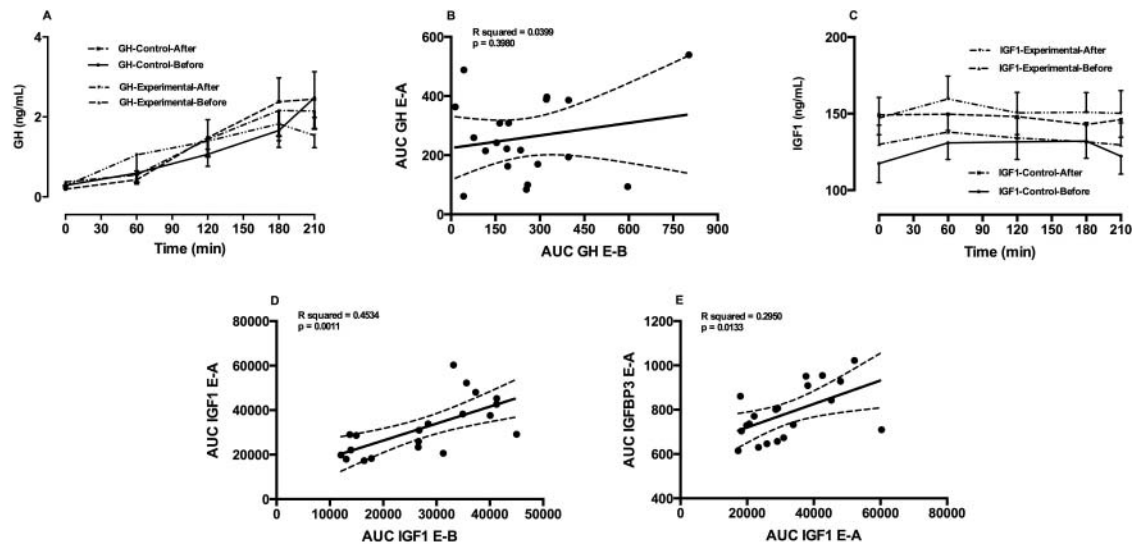
### IGF1 Analysis

Plasma IGF1 increased at the end of study in both the control and experimental groups during venous zinc administration (Fig. 3C). However, the AUC of IGF1 increased significantly only in the experimental group, with a positive correlation (Fig. 3D). However, the AUC of IGF1 did not show any difference between both groups studied (data not shown). Additionally, there was a positive correlation between the AUC of IGF1 and the AUC of IGFBP3 in the experimental group (Fig. 3E) and no correlation with the AUC of OCN. The concentrations of IGF1 were within the normal range.

### IGFBP3 Analysis

Plasma IGFBP3 concentrations increased in the control and experimental groups after venous zinc administration (Fig. 4A). The AUC of IGFBP3 was significant only in the experimental group (*p* < 0.0001). In addition, there was a

# Effect of Zinc on GH-IGF1 System in Children

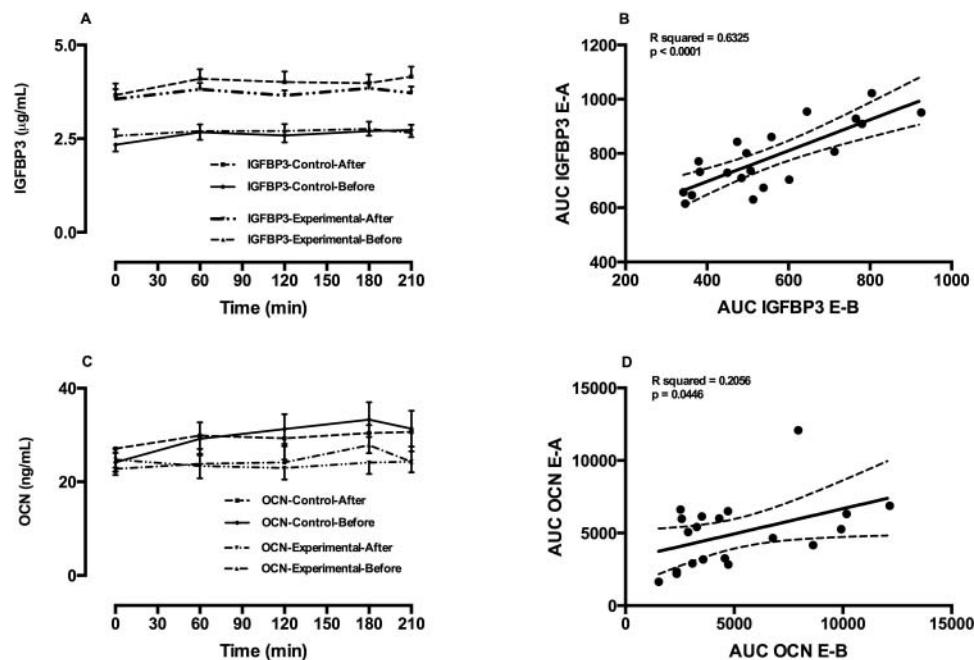


**Fig. 3** (A) Plasma GH in control and experimental groups; (B) correlation between AUC of GH before and after oral zinc supplementation in the experimental group; (C) plasma IGF1 in control and experimental groups; (D) correlation between AUC of IGF1 before and after oral zinc supplementation in the experimental group; (E) correlation between AUC of IGF1 and AUC of IGFBP3 before and after oral zinc supplementation in the experimental group. Notes. E-B = experimental before; E-A = experimental after. Values are expressed as the mean  $\pm$  SEM, R squared and  $p$ . A  $p$  value of  $< 0.05$  was considered significant.

positive correlation between the AUC of IGFBP3 in the experimental group (Fig. 4B). However, there was no correlation between the AUC of IGFBP3 and the AUC of OCN in the experimental group (data not shown). The concentrations of this hormone were within the normal reference range.

## OCN Analyses

As with other hormones, OCN also increased during venous zinc administration after oral zinc supplementation (Fig. 4C). The analysis of the AUC of OCN showed no statistical significance in the control and experimental groups or between them.



**Fig. 4** (A) Plasma IGFBP3 in control and experimental groups; (B) correlation between AUC of IGFBP3 before and after oral zinc supplementation in the experimental group; (C) plasma OCN in control and experimental groups; (D) correlation between AUC of OCN before and after oral zinc supplementation in the experimental group. Notes. E-B = experimental before; E-A = experimental after. Values are expressed as the mean  $\pm$  SEM, R squared and  $p$ . A  $p$  value of  $< 0.05$  was considered significant.

However, a positive correlation was observed in the experimental group (Fig. 4D). Their plasma concentrations were within the normal reference range.

### Hematological and Biochemistry Analysis

The hematological analyses were within the normal reference ranges (data not shown). The serum concentrations of total proteins showed no differences in the control group and the experimental group, as well as between these 2 groups. However, serum concentrations of ALP were significant only in the experimental group ( $p = 0.0270$ ). Serum TRAP did not change in the control and experimental groups or between them (data not shown). All parameters were within the normal reference ranges.

## DISCUSSION

### Anthropometric Assessment

Weight, height, and BMI for age increased at the end of the study in experimental group. These results were expected from physiological and pharmacological points of view [7]. Though a positive correlation between BMI and serum IGF1 was reported before zinc supplementation in children with zinc deficiency [17], we did not find any correlation, either before or after zinc supplementation. On the other hand, the increase in serum IGF1 concentration was higher in 29 zinc-deficient children with low BMI after zinc supplementation [17], although other researchers reported that IGF1 was unaffected by BMI [16]. Additionally, we did not observe any correlation between BMI and GH, IGFBP3, or OCN.

### Dietetic Assessment

Only schoolchildren in the experimental group showed increased values for energy, protein, and total fat intake after oral zinc supplementation. Similar results were obtained by Alves et al. [7], although Leite et al. [35] reported no positive effect of zinc on macro- and micronutrients. The values of calcium intake were lower than the estimated average requirement [33], which could contribute negatively to growth. Despite this factor, a significant increase was observed in the experimental group,  $p < 0.0001$ . The same result was previously reported by other researchers [7,35]. Regarding zinc, the value of the tolerable upper intake level was higher in the experimental group after oral zinc supplementation than in the control and experimental groups before oral zinc supplementation ( $p = 0.0353$  and  $p < 0.0001$ , respectively), which shows a positive change in the zinc status after oral administration. The relationship between zinc and growth is too close because Zip1 and Zip2 mRNA expression positively correlated with growth hormone level and was higher in short children [9]. Moreover, a study

with 795 preadolescent children showed that increased consumption of dairy products is capable of improving height due to the rs680 IGF2 genotype [36]. Although we have found a lower intake of fiber (Table 2), this macronutrient slightly influences the bioavailability of zinc. However, concomitant poor dietary intakes of energy, calcium, and zinc contribute to impaired bone metabolism [37]. Regarding GH, IGF1, IGFBP3, and OCN, we did not observe any correlation between energy intake and these hormones, and results from other studies were not available for us to compare our findings.

### Zinc Analysis before and after Oral Zinc Supplementation

Basal serum zinc concentrations after oral zinc supplementation increased significantly in the experimental group and this result was expected and corroborated by Alves et al. [7] and Leite et al. [35]. Additionally, other authors reported the positive effect of zinc intake and/or zinc supplementation on serum zinc status depending on the duration of intervention, dose of supplementation, and nutritional situation [5,38].

### Zinc Analysis before and after Venous Zinc Administration

The profiles of serum zinc and the AUC of zinc increased with venous administration, consistent with the results observed by Alves et al. [7] and Leite et al. [35]. There was a positive correlation between the AUC of zinc in the experimental group and a negative correlation between the AUC of zinc and the AUC of GH in this group. This result is contradictory to the reports of other authors, who showed a positive correlation between the AUC of zinc and the AUC of GH after oral zinc supplementation in 30 normal children [7]. Additionally, we did not observe any correlation between the AUC of zinc and the AUC of IGF1, IGFBP3, and OCN. In this sense, no correlation was also observed between zinc and IGF1 and IGFBP3 after zinc supplementation [7], although a correlation was observed by other researchers [17]. On the other hand, there are no reports in the literature of this type of correlation with OCN.

### GH Analysis

In the present study, we found a significant increase in GH secretion during intravenous zinc administration in the control and experimental groups, suggesting action of this micronutrient (Fig. 3A). A similar increase was also observed in healthy children supplemented with zinc [7] and, conversely, no effect was observed on plasma GH and the expression of growth hormone receptor mRNA in male KunMing mice [4]. However, the fact that we have not found differences in the analysis of AUC of GH and correlations of GH before and after placebo or oral zinc supplementation, this does not contradict GH secretion during the injection of zinc. For instance, a positive



correlation was observed between the AUC of GH and the AUC of zinc after oral zinc supplementation in 30 prepubertal children [7]. Fifty prepubertal Egyptian children with short stature and zinc deficiency presented lower GH peaks after insulin and clonidine stimulation and no difference was observed before and after oral zinc supplementation, a result very similar to ours in healthy children [39]. Corroborating ours results, oral zinc supplementation ameliorated growth retardation in children without zinc deficiency [40]. GH secretion depends on the aggregation of its molecules in the biosynthesis of secretory granules, a process dependent upon zinc ions [41]. In this regard, these authors report the role of zinc transporters of Slc30a/ZnT and Slc39a/Zip families in the control of GH secretion. Moreover, loss of affinity of GH to zinc or a decrease in free zinc content in the secretory granules could interfere with normal GH secretion [13].

### IGF1 Analysis

Zinc deficiency can seriously impair growth in children and adolescents, especially because it affects the synthesis and secretion of IGF1. We did not observe any correlation between the AUC of IGF1 and the AUC of zinc, although other authors have detected this correlation before oral zinc supplementation [17,39]. Additionally, low serum IGF1 concentrations were detected in short children with or without zinc deficiency, which increased after supplementation of 50 mg Zn/day for 3 months [3,15,17,39]. Continuous infusion of GH normalized the liver growth hormone receptor, whereas serum IGF1 and its liver mRNA were not stimulated by this hormone in zinc-deficient rats. This indicated that the presence of this micronutrient in this type of hormonal reaction is necessary [42]. On the other hand, zinc deficiency decreases IGF1 concentration independent of total energy intake [43] and, in this sense, we did not find any correlation between BMI and total energy intake with IGF1.

### IGFBP3 Analysis

We did not observe any correlation between the AUC of IGFBP3 and the AUC of zinc, similar to Alves et al. [7], although stimulatory effect of zinc on serum IGFBP3 was observed in short children without zinc deficiency [3]. Circulating IGFBP3 concentrations are influenced by age, sex, height, and BMI. In experimental animals, zinc deficiency caused alterations in the plasma concentrations of IGFBP3 [44] and when submitted to infusion of bovine GH there was normalization of circulating IGFBP3 [42]. We did not observe any correlation between BMI and total energy intake with IGFBP3. This close relationship between IGFBP3 and BMI and energy is not well understood, and it is critical to ascertain the nutritional status of children [45].

### OCN Analysis

We did not observe any correlation between the AUC of OCN and the AUC of zinc. However, the interrelationship between zinc and OCN was studied by Yamaguchi and Hashizume [46], who demonstrated that  $\beta$ -alanyl-L-histidinato zinc was markedly effective in increasing this hormone in the culture medium secreted from osteoblastic cells. Additionally, zinc supplementation was effective in stimulating serum OCN in non-zinc-deficient idiopathic short children [3]. Another study showed that dietary zinc intervention could increase markers of osteoblast differentiation, matrix maturation, and mineralization in the long bones of growing rats. Among these parameters, zinc positively increased the maximum expression of OCN [18]. Moreover, OCN levels were lower for patients with diabetes with zinc intake levels below the recommended dietary allowance and there was a close correlation between them [47]. However, a correlation between BMI and total energy intake with OCN was not detected in our study.

### Hematological and Biochemical Analysis

Hematological tests were normal in our sample. The biochemical parameters, as total proteins and acid phosphatase, were unchanged in both groups in our study. However, alkaline phosphatase increased in the children supplemented with zinc, most likely indicating that this protein is most sensitive to small changes in the concentration of zinc in the blood even in healthy and eutrophic children [3].

Taken together, growth is dependent on GH, IGF1, and IGFBP3, and the exact mechanism by which zinc acts on this system is not well understood. Moreover, this complexity lies in the fact that zinc also acts positively or negatively on the metabolism of vitamin D, testosterone, estrogen, and thyroid hormones, and insulin. It is important to determine the nutritional status of children because the hormones mentioned above are dependent on good nutrition. Additionally, in this context, the status of zinc should be systematically evaluated because zinc deficiency can affect the effect of GH treatment in children with short stature, which is reversible with supplementation of this micronutrient.

Our study was characterized as a convenience sample, and we used sample size calculation for comparing 2 means (paired samples) as follows:  $n = (Z\alpha + Z\beta)^2 \cdot \sigma^2 D / \delta^2$ . The sample size of 40 schoolchildren was adequate for the conclusions found in this study because for any value  $\delta \geq -0.09$ . Additionally, the sample size required was  $n = 15$ .

## CONCLUSIONS

We report that oral zinc supplementation (1) increased the total energy, protein, and fat intake without a correlation between BMI and total energy intake with oral zinc

supplementation and hormones studied; (2) increased the basal serum zinc; (3) increased the plasma alkaline phosphatase; and (4) showed a positive correlation for IGF1, IGFBP3, and OCN, comparing before and after oral zinc supplementation. Moreover, zinc administration increased the serum zinc and plasma GH, IGF1, and IGFBP3 in the experimental group.

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