



Research Article

## Zinc Supplementation Increases Food Intake and HDL-c and Decreases Platelets in Healthy Children

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### Abstract

**Objective:** The aim of the present study was to evaluate the effects of oral zinc supplementation on food intake, and biochemical and hematological parameters in healthy children.

**Methods:** Fifty children of both genders, aged 8-9 years, were studied during a three-month period. The study was a randomized, controlled, triple-blind study that used non-probability sampling. The children were randomly assigned to the control (n=25, using placebo) and experimental (n=25, using 10 mg/day elemental zinc) groups.

**Results:** Both the control and experimental groups showed significant increases in weight, height, body mass index, and intake of all macro- and micronutrients after 3 months of participation. The intake of energy (p<0.0004), fat (p<0.0001), and protein (p<0.0001) increased significantly after oral zinc supplementation compared to oral placebo. Basal serum zinc levels increased significantly in the control (p<0.0001) and experimental (p<0.0001) groups at the end of the study. However, in the experimental group, alkaline phosphatase (p=0.0232) and high-density lipoprotein cholesterol (HDL-c) (p<0.0001) levels increased significantly and platelet count decreased (p=0.0014) significantly after oral zinc supplementation, although neither variable showed a correlation with serum zinc.

**Conclusions:** Zinc supplementation contributed to significant increases in energy intake, fat and protein consumption in healthy children. Importantly, zinc increased the HDL-c levels and decreased the platelet count.

### Keywords

Zinc supplementation; Triple-blind study; Biochemical parameters; Hematological parameters; Healthy children

### Introduction

Zinc is an essential trace element in humans and animals and has remarkably diverse biological effects [1]. Zinc-dependent enzymes span all enzyme classes and participate in a variety of metabolic processes, including the synthesis and/or degradation of lipids, carbohydrates, proteins and nucleic acids [2-4].

Nutritional zinc supplementation is used for the prevention and treatment of diabetes mellitus, growth deficiency, and many infectious diseases [5]. Comparisons of studies on zinc supplementation are frequently difficult because of different methodological designs, interactions with other nutrients, and confounders such as unawareness of the previous nutritional status of participants [5].

Food intake, consisting of energy, macronutrients, fiber, calcium, iron, and zinc, can change due to the action of zinc, likely because children supplemented with zinc ingest more calories [6]. However, suboptimal energy consumption has been reported in children while fat intake was unchanged [7], and protein intake has been reported to increase significantly after oral zinc supplementation [8]. The consumption of carbohydrates and fiber did not change after zinc supplementation [7]. Some authors have reported increases [9] or no significant changes [7] in the consumption of calcium and iron after zinc supplementation. For zinc, there are reports showing increases [8] or no change in its consumption after oral zinc supplementation [7].

Zinc influences thrombogenesis and hemostasis via platelet aggregation and coagulation [10,11]. Zinc depletion impairs platelet aggregation and prolongs bleeding times in adult males [12], while zinc supplementation restores the functional integrity of platelets [13]. By contrast, hyperzincemia per se, after 50 mg Zn/day, has also been shown to increase platelet reactivity [10].

Regarding zinc and alkaline phosphatase, this interrelationship is well studied in the literature and this hydrolase enzyme is a byproduct of osteoblast activity, associated with bone formation [14,15]. However, alkaline phosphatase activity did not appear to be effective biomarkers of zinc status [14].

The interrelationship between zinc and plasma lipids is contradictory: studies have shown both a positive association and no association. For instance, high-density lipoprotein cholesterol (HDL-c) levels were reduced after intake of higher doses of zinc [16] or were unchanged with lower doses [17]. Importantly, almost all of these studies were performed in healthy adults rather than in children. Although high doses of zinc may decrease the plasma levels of HDL-c, contributing to increased risk of coronary heart disease, moderate zinc doses of 15 mg/d may be considered non-detrimental to health [18]. Regarding low-density lipoprotein cholesterol (LDL-c), Foster et al. [19], using meta-analysis, reported that LDL-c levels were not changed by zinc supplementation in the overall and ungrouped analyses. Similar results were observed in the sub-analyses when interventions were categorized by gender, duration, zinc anion, and whether zinc was provided alone or in combination with other supplements [19]. However, there have been reports showing increased LDL-c levels when doses of 30 mg Zn/day were used in middle-aged individuals [18]. Total cholesterol (TC) was also increased by zinc intervention [18] or was shown to be unchanged in the overall, ungrouped or grouped analyses [19]. Additionally, triglycerides (TGs) are only minimally affected by zinc [17,19]. Interestingly, the physiological levels of fatty acids modulate the zinc-binding capacity of albumin, which is important for the biological speciation of zinc and for its clinical significance [20].

In view of conflicting results in the literature, the aim of this study was to analyze whether oral zinc supplementation in physiological dose could change food intake, biochemical and hematological parameters in healthy children.

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Received: September 23, 2015 Accepted: December 03, 2015 Published: December 08, 2015

## Materials and Methods

### Subjects

Fifty young children of both genders, aged 8-9 years, from three municipal schools of Natal City, RN, Brazil, were included in the study. The students were authorized by their parents or guardians to participate in the study. 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). The Universal Trial Number (UTN) is U1111-1169-3107.

### Selection criteria

The study included only children who were apparently healthy based on medical, anthropometric and laboratory evaluations. All children were in the Tanner stage 1 for genital, breast, and pubic hair growth [21] and had body weight, height, and body mass index (BMI) within the normal reference range for their ages [22]. Exclusion criteria included basal serum zinc below 0.7 µg/mL; early pubarche, thelarche or menarche; acute, chronic, infectious or inflammatory diseases; and children who had undergone surgery or were using vitamin and mineral supplements.

### Experimental design

The children were examined and studied for 3 months at the Laboratory of Multidisciplinary Chronic Degenerative Diseases at UFRN. It was a randomized, controlled, triple-blind study that employed a process of non-probability sampling in which neither the subject nor the person administering the treatment nor the person evaluating the response to treatment knows which treatment any particular subject is receiving. The subjects were recruited via an advert on school noticeboards and meetings with parents. The control group was comprised of 25 children (14 males and 11 females) who used a placebo. The experimental group was comprised of 25 children (13 males and 12 females) who used 10 mg/day elemental zinc by oral administration, and the pairing was done randomly. Parents and children did not know which oral solution was received as well as did not know which group they belonged. Only one member of the team controlled the experiments, only revealing which children belonged to the control or experimental group at the time of the data collection.

Serum zinc was observed before and after 3 months of zinc supplementation. Blood collection began at 7:00 am and ended at 8:00 am after a 12 hr overnight fast. Venipuncture from an antecubital forearm vein was performed using plastic, metal-free syringes without a tourniquet. The placebo or zinc solutions were not administered on the days of these blood collections. Zinc vials and zinc intake were monitored every two weeks by the same nutritionists during home visits. A medical doctor performed a history and physical examination, and nutritionists performed an anthropometric and nutritional assessment.

### Anthropometric assessment

Body weight (kg) and height (cm) were measured using an electronic balance (Balmak, BK50F, São Paulo, SP, Brazil) and a stadiometer (Sanny Stadiometer Professional, American Medical of Brazil, São Paulo, SP, Brazil), respectively. The assessment of nutritional status was based on anthropometric indicators (weight-for-age, height-for-age, and body mass index-for-age (BMI-for-age)). The analysis of nutritional status was also based on BMI-for-age, based on the growth curves published by the World Health

Organization [22]. We used an online program to calculate BMI-for-age [23]. To measure weight, the child remained standing on the balance without shoes and wearing light clothing. To measure height, the child remained standing without shoes, with heels together and the body as straight as possible. The heels, buttocks, shoulders and head touched the vertical surface of the measuring equipment.

### Dietetic assessment

Food intake evaluation was performed with a prospective 3-day food record on two weekdays and one weekend day. The parents were instructed to record all food and beverages consumed by the child using household measures. Calculations of energy, macronutrients, fiber, calcium, iron and zinc were performed using NutWin software, version 1.5 [24]. Foods not included in the program were inserted based on food chemical composition tables [25], and all data were analyzed based on dietary reference intakes (DRIs) and estimated average requirements (EAR) [26-29].

### Oral placebo

The control group received an oral placebo (10% sorbitol) under the same conditions as the oral zinc solution.

### Oral zinc supplementation

The experimental group received 10 drops of zinc solution (10 mg Zn/day) as zinc sulfate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O, Merck, Darmstadt, Germany), added to milk or juice every morning at breakfast. Syrups were prepared at the Pharmacotechnical Laboratory of the Department of Pharmacy at UFRN. Zinc ingestion was controlled every two weeks by the same observers who performed the previous measurements.

### Handling of zinc

Blood (4 mL) was collected for analysis of serum zinc (BD Vacutainer, Trace Element, Serum, Franklin Lakes, NJ, USA). All material used for the collection, separation and storage of zinc was metal-free polypropylene. The tubes were purchased from Becton Dickinson (Franklin Lakes, NJ, USA), and the pipettes were purchased from Bio-Rad (Hercules, CA, USA). The micronutrient samples were handled according to international standards [30]. We used a stainless steel incubator (502, Fanem, São Paulo, SP, Brazil) to form clots. A serum (500 µL) dilution was made with ultrapure water (2 mL) (Milli-Q Plus, Millipore, Billerica, MA, USA) and stored at -80°C for subsequent analysis of zinc, in triplicate, by atomic absorption spectrophotometry (SpectrAA-240FS, Varian, Mulgrave, Victoria, Australia) according to the manufacturer's instructions. The standard zinc solution (1,000 mg/mL) was obtained by diluting standard zinc Titrisol (Merck, Darmstadt, Germany) in ultrapure water. The sensitivity of zinc measurements was 0.01 µg/mL, the coefficient of variation was 2.37%, and the reference interval was 0.7-1.2 µg/mL according to our laboratory.

### Biochemical and hematological parameters

Glucose, total bilirubin, total protein, urea, uric acid, TGs, TC, HDL-c, VLDL-c, LDL-c, alkaline phosphatase, and TRAP were measured using a colorimetric method in a biochemical analyzer with specific kits (Dade Behring Dimension AR, Deerfield, Illinois, USA). Hematocrit, hemoglobin, mean corpuscular volume, and eosinophils were measured using standard clinical laboratory methods with specific kits (Horiba ABX Diagnostics, Micros 60, Montpellier, France).

## Statistical analyses

Statistical analyses included the D'Agostino-Pearson omnibus normality test to analyze the normality of all study data. Paired and unpaired Student's *t* tests were used to compare the data obtained within the control and experimental groups or between the two groups. Wilcoxon matched-pairs signed rank test was used to complement paired nonparametric test and Mann-Whitney test to complement unpaired nonparametric test. Comparisons between two variables per subject were measured using Pearson correlation coefficients and Spearman's test for nonparametric correlations. Tukey's multiple comparison test was used to compare every mean with every other mean within the control and experimental groups. To verify whether the results were accurate for the population studied, we performed a sample size calculation for comparing two means (paired samples) as follows:  $n = (Z\alpha + Z\beta)^2 \cdot \sigma_D^2 / \delta^2$ . The selected level of significance was  $p = 0.05$ . Statistical tests were performed using GraphPad Prism 6.0 (San Diego, CA, USA).

## Results

### Subjects

The children were in Tanner stage 1, and chronological age and gender are shown in Table 1. The children in the control and experimental groups presented with homogeneous anthropometric (Table 1) and food intake (Figure 1 and Table 2) characteristics. The sample size of 50 schoolchildren was adequate for the conclusions obtained in this study given that for any value of  $\alpha = 0.05$ ,  $\sigma^2 = 0.105034$  and  $\delta = -0.09$ , the sample size required would be  $n = 15$ . There was no sample loss.

### Anthropometric and body composition assessments

Both the control group and the experimental group presented significant increases in weight, height, and BMI after three months. These parameters did not show any correlation with oral zinc supplementation in the experimental group. All schoolchildren had adequate anthropometric nutritional status during the study (Table 1).

### Dietetic assessment

The intake of all macro- and micronutrients increased significantly in both the control and experimental groups. Calcium intake increased only in the experimental group (Table 2).

Comparisons between the control group (after placebo) and experimental group (after zinc) showed that the energy, fat, and protein intake increased more significantly after oral zinc supplementation than with oral placebo. However, the intake of carbohydrate, fiber, calcium, iron and zinc showed no significant differences between the placebo and zinc supplementation groups at the end of the study

(Figure 1 and Table 2).

Total zinc intake (dietary + oral supplemental) exceeded 12 mg Zn/day, which is the maximum zinc intake (tolerable upper zinc intake level) recommended for children between 4 and 8 years old (Figure 2A) and did not exceed 23 mg Zn/day, which is the maximum zinc intake (tolerable upper zinc intake level) for children between 9 and 13 years old (Figure 2B) in the experimental group. However, there was no significant difference between the ages of the children in the two groups ( $p = 0.7234$ ), and there were no adverse effects of zinc supplementation. Additionally, the intake of all macro- and micronutrients did not show any correlation with serum zinc or the maximum zinc intake level (tolerable upper zinc intake level) in the experimental group.

### Oral placebo

The control group showed a significant increase in the basal serum zinc level at the end of study, showing a positive correlation between before and after placebo supplementation (Figure 3A and 3B).

### Oral zinc supplementation

Basal serum zinc levels increased significantly after oral zinc supplementation. There was a positive correlation between before and after oral zinc supplementation (Figure 3C and 3D). There was no difference between the basal serum zinc in the control group after placebo and the basal serum zinc in the experimental group after zinc supplementation ( $p = 0.8629$ ).

### Biochemical parameters

Glucose, total bilirubin, total protein, urea, uric acid, TGs, TC, HDL-c, VLDL-c, LDL-c, and TRAP did not change in the control and experimental groups. However, alkaline phosphatase increased significantly in the experimental group after oral zinc supplementation ( $p = 0.0232$ ), without correlation with serum zinc (data not shown). HDL-c also increased significantly in the experimental group ( $p < 0.0001$ ), although it did not correlate with serum zinc (Figure 4A and 4B).

### Hematological parameters

Hematocrit, hemoglobin, mean corpuscular volume, and eosinophils did not change in either the control or experimental groups. However, platelet count decreased significantly in the experimental group ( $p = 0.0014$ ), with no correlation with serum zinc after oral supplementation (Figure 4C and 4D).

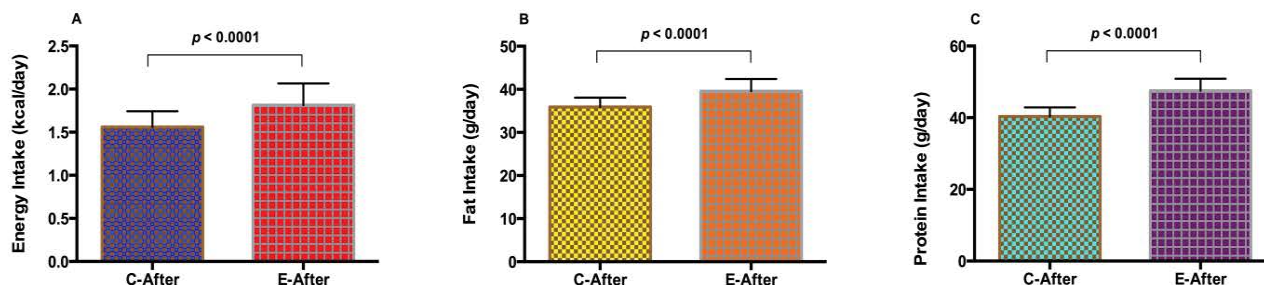
## Discussion

This is a study in healthy children, who are not often the subjects

**Table 1:** Values of anthropometric and body composition obtained before and after oral zinc administration in 50 apparently healthy children.

Parameter	Control (n=25)			Experimental (n=25)		
	Before	After	p value	Before	After	p value
Age (y)	8.69 ± 0.52	---	---	8.84 ± 0.52	---	---
Gender						
Male	n=14	---	---	n=13	---	---
Female	n=11	---	---	n=12	---	---
Weight (kg)	27.75 ± 4.17	28.72 ± 4.80	< 0.0001*	26.71 ± 4.60	27.73 ± 4.82	< 0.0001*
Height (cm)	131.70 ± 6.36	133.00 ± 6.48	< 0.0001*	130.30 ± 4.80	131.80 ± 4.97	< 0.0001*
Body mass index (kg/m <sup>2</sup> )	15.93 ± 1.68	16.16 ± 1.95	0.0400*	15.65 ± 1.91	15.86 ± 1.92	0.0081*

Values are expressed as the means ± SD and p values with superscripts are statistically significant at  $p < 0.05$ .

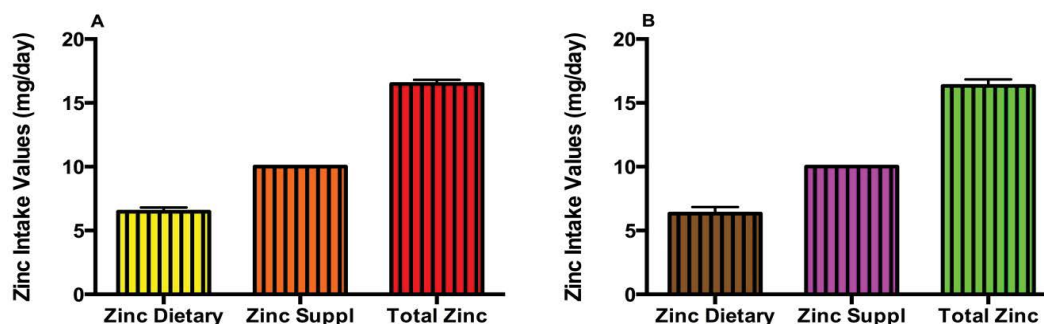


**Figure 1:** Values for energy intake (A), fat intake (B), and protein intake (C) obtained after oral placebo (control group) and after oral zinc supplementation (experimental group) in 50 apparently healthy children. C-After means control group after oral placebo. E-After means experimental group after oral zinc supplementation.

**Table 2:** Values of food intake obtained before and after placebo or zinc supplementation in 50 apparently healthy children.

Parameter	Control (n=25)			Experimental (n=25)		
	Before	After	p value	Before	After	p value
Energy (kcal/day)	1551 ± 0.18	1560 ± 0.18	0.0079 <sup>*</sup>	1622 ± 0.23	1805 ± 0.27	< 0.0001 <sup>*</sup>
Carbohydrate (g/day)	181.90 ± 10.83	180.80 ± 10.84	< 0.0001 <sup>*</sup>	181.40 ± 19.95	178.90 ± 19.91	< 0.0001 <sup>*</sup>
Fat (g/day)	35.72 ± 2.17	35.89 ± 2.18	0.0083 <sup>*</sup>	35.85 ± 2.02	39.41 ± 2.95	< 0.0001 <sup>*</sup>
Fiber (g/day)	10.63 ± 1.07	11.09 ± 1.04	< 0.0001 <sup>*</sup>	10.15 ± 1.02	11.42 ± 0.95	< 0.0001 <sup>*</sup>
Protein (g/kg/day)	40.00 ± 2.35	40.31 ± 2.55	0.0061 <sup>*</sup>	42.00 ± 3.62	47.51 ± 3.43	< 0.0001 <sup>*</sup>
Calcium (mg/day)	634.80 ± 106.80	627.20 ± 92.09	0.9423	581.00 ± 67.28	637.60 ± 59.53	< 0.0001 <sup>*</sup>
Iron (mg/day)	8.87 ± 0.75	9.04 ± 0.69	0.0038 <sup>*</sup>	8.73 ± 0.41	9.31 ± 0.41	< 0.0001 <sup>*</sup>
Zinc (mg/day)	6.16 ± 0.48	6.47 ± 0.45	< 0.0001 <sup>*</sup>	5.82 ± 0.43	6.39 ± 0.44	< 0.0001 <sup>*</sup>

Values are expressed as the means ± SD and p values with superscripts are statistically significant at p<0.05.



**Figure 2:** Total zinc intake (dietary + oral supplemental) in 25 apparently healthy children in the experimental group. (A) Children of 8 years old. (B) Children of 9 years old. Suppl = supplementation.

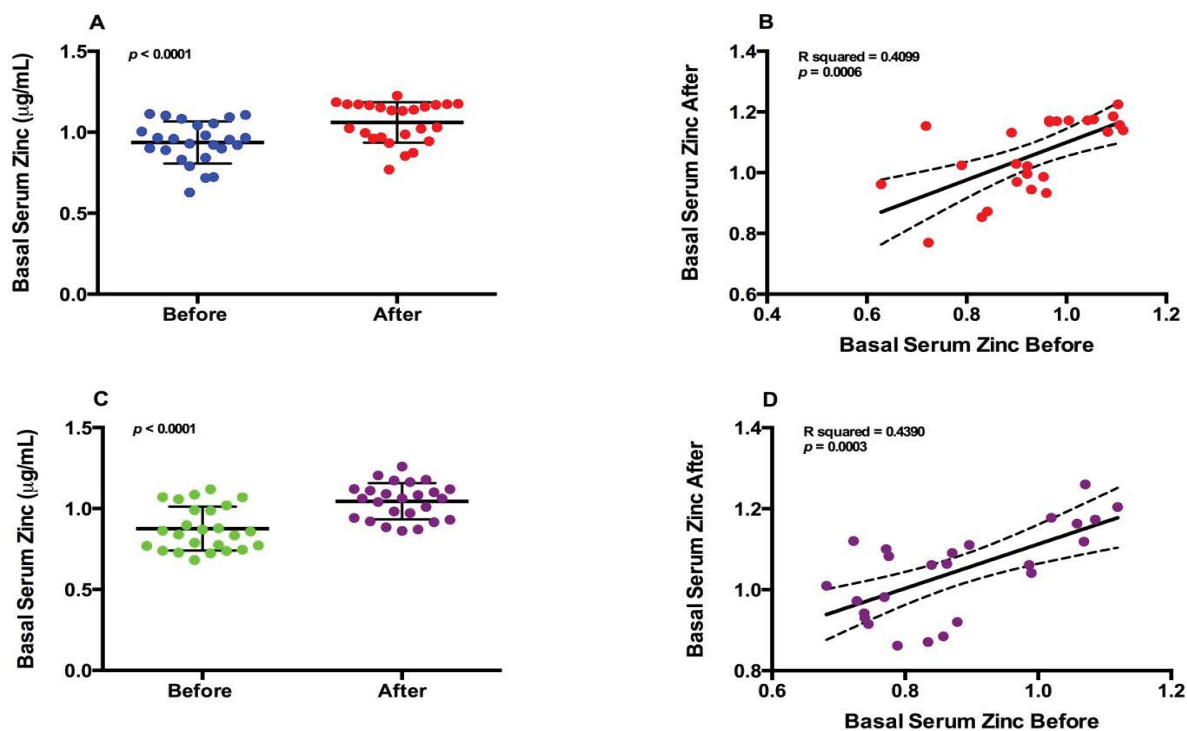
of research studies. This work shows that oral zinc administration can change the levels of food intake, HDL-c and platelets.

Significant increases in weight and height ( $p < 0.0001$ ) were observed in both the control and experimental groups. Every child grows during childhood and adequate dietary intake is essential for growth and zinc supplementation has been shown to accelerate gains in weight and height in children either with [31] or without zinc deficiency [9]. However, in our experimental group, these parameters showed no correlation with zinc after oral supplementation, although correlation is not evidence of causality. Similarly, no correlation between basal serum zinc levels with these anthropometric parameters was reported in children aged 6 months to 24 months [32]. However, a positive correlation was observed between zinc intake and weight and height in children aged 3-6 years [33].

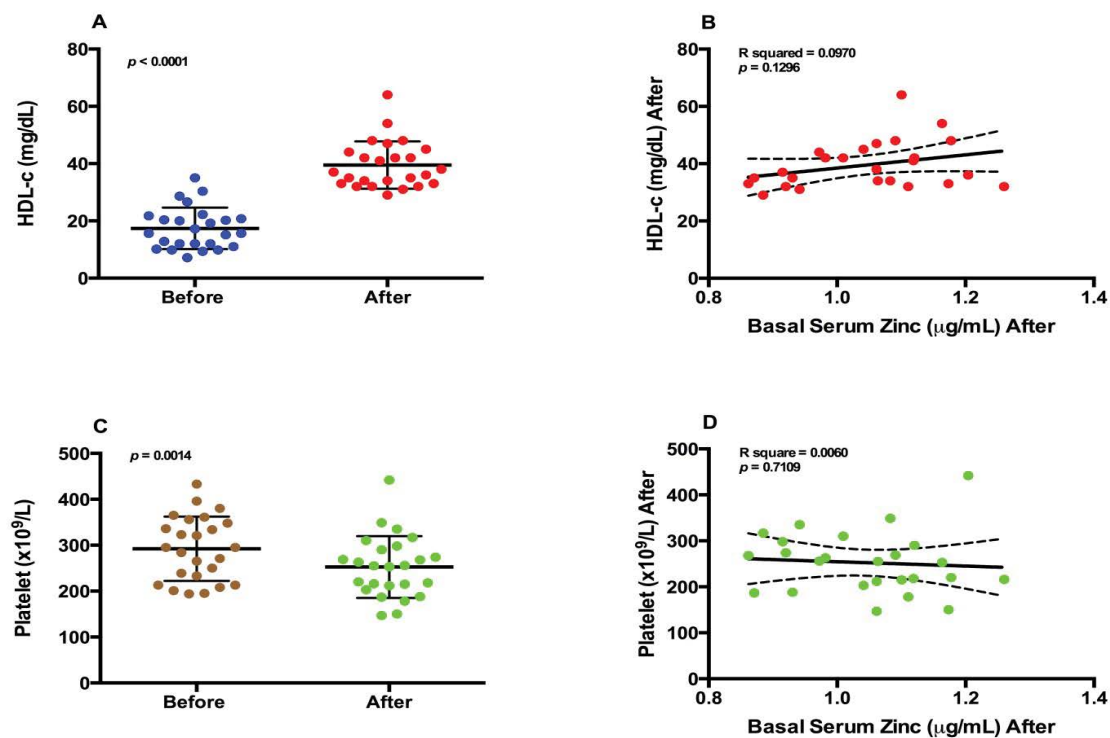
In the present study, the BMI also significantly increased in

both the control and experimental groups, although BMI showed no correlation with zinc after oral supplementation in the experimental group. This result was consistent with results reported in adults by other authors [31,34], although Bae and Cho [35] and Lopes et al. [36] reported the opposite. Therefore, the children in our study had adequate anthropometric nutritional status.

Energy intake increased in both the control and experimental groups. However, the increase was more significant in the experimental group ( $p < 0.0001$ ) comparing with the control group. This is likely because children supplemented with zinc ingest more calories [6]. Interestingly, Yu [33] reported a positive correlation between serum zinc levels and levels of calorie intake. Intake of the macronutrients carbohydrates, fat, fiber, and protein increased in both the control and experimental groups. However, only the consumption of fat and protein were significantly higher in the experimental group after oral zinc supplementation compared with the control group after oral



**Figure 3:** Basal serum zinc concentrations (A) and correlation (B) before and after placebo supplementation in the control group. Basal serum zinc concentrations (C) and correlation (D) before and after zinc supplementation in the experimental group.



**Figure 4:** Concentrations of HDL-c (A and B) and platelets (C and D) before and after zinc supplementation in the experimental group.

placebo supplementation ( $p < 0.0001$ ). In other studies, fat intake was not significantly different before and after zinc supplementation, remaining constant over 3 months [7-9]. Regarding protein, there are reports in the literature showing either increases [8,9] or non-significant differences after zinc supplementation [7]. In our study, the consumption of carbohydrates and fiber did not differ significantly between the experimental group after oral zinc supplementation and the control group. Thus, the consumption of these macronutrients was essentially unchanged by zinc supplementation [7,8], and a positive correlation was observed between serum zinc levels and fiber intake [33].

The levels of calcium, iron, and zinc consumption were also not significantly different between the experimental group after oral zinc supplementation and the control group. There are reports in the literature showing either increases [9,36] or no significant differences in calcium and iron intake [7,8] after zinc supplementation. Additionally, serum zinc levels correlated positively with the levels of calcium and iron intake [33]. Regarding zinc consumption, there are reports of an increase [8,9] or no change in consumption after oral zinc supplementation [7]. However, there was no correlation between zinc intake and plasma zinc levels in children aged 6-24 months [32]. Similar results were observed for comparisons between total zinc intake and serum zinc levels in children 6-11 years old [37], in children aged  $7.0 \pm 0.5$  years [38] or among adolescents aged  $13.2 \pm 1.0$  years [39]. Conversely, positive correlations between total zinc intake and serum zinc levels were reported for girls aged 4-18 years [40] and for adults aged 20-65 years [41].

Moreover, to the best of our knowledge, there are no reports in the literature correlating tolerable upper zinc intake levels with the intake of energy, carbohydrate, fat, fiber, protein, calcium, or iron.

In our study, basal serum zinc levels increased in the control group after placebo treatment. A similar result was observed by other authors [36,37], and it appears to be related to the increase in energy consumption by the children. Similar results obtained with oral placebo were found in the basal serum zinc levels after zinc supplementation, and these results were corroborated by other authors [37,42,43]. Zinc supplementation of 10 mg/day plus zinc intake did not exceed the maximum recommended zinc intake in children between 9 and 13 years. No side effects were observed at the aforementioned dose of zinc.

The synthesis of alkaline phosphatase is impaired in children with zinc deficiency and increased in the children supplemented with zinc, indicating that this protein is most sensitive to small changes in the levels of zinc even in healthy children [15]. Lowe et al. [14] reported no significant effect of zinc intakes on plasma alkaline phosphatase activity, which suggests that this is not a useful zinc biomarker. However, our results showed an increase in alkaline phosphatase activity.

HDL-c was the only lipid parameter that increased significantly in the experimental group after oral zinc supplementation, although this was not correlated with serum zinc. To the best of our knowledge, this is the first time this result has been reported in healthy children. HDL-c levels decrease during the first half of adolescence in boys, and changes in body fat have shown negative associations with HDL-c levels [44]. Although there are many reports addressing HDL-c levels in adults, they are scarce in children. Thus, Foster et al. [19], using meta-analysis, reported no change in HDL-c levels after zinc supplementation when the interventions were grouped according

to gender, zinc dosage, zinc anion, zinc administered alone or with other supplements, or trial duration. Instead, the effects of zinc were only observed when interventions were grouped according to the age of the participants. Individuals aged  $\leq 40$  years showed significantly decreased HDL-c levels, whereas individuals aged 40-55 years showed increased HDL-c levels, and those  $\geq 55$  years showed no significant changes in plasma HDL-c levels [19]. In this context, our results are interesting because we observed increases in plasma HDL-c and normal LDL-c levels, which are considered useful parameters for predicting dyslipidemia and subclinical atherosclerosis [45]. The fact that we have not observed changes in plasma LDL-c, total cholesterol, and triglycerides after zinc intervention is consistent with the expected results for adults in the medical literature [17,19]. In this context, the nutraceutical and functional food ingredients containing zinc could be used to prevent or treat HDL-c deficiency [46]. This is an aspect that should be taken into account because zinc can enhance paraoxonase enzyme activity in HDL, and is involved in the prevention of LDL oxidation [47,48]. Furthermore, this process could reduce the incidence of cardiovascular disease [49].

Hematocrit, hemoglobin, mean corpuscular volume, and eosinophils did not change in either the control or experimental groups. These results are corroborated by other authors [8,50,51], and they indicate that doses of 10-20 mg Zn/day represent a safe intervention in apparently healthy children aged 0-15 years. In our study, platelet count decreased significantly in the experimental group, although this decrease did not correlate with serum zinc. Although we have not tested platelet function, there are studies specifically demonstrating the importance of zinc in modulating key signaling events that influence platelet aggregation. In this regard, Vu et al. [11] summarized the effects of zinc as follows: (1) zinc enhances platelet aggregation, inducing the production of collagen and adenosine diphosphate (ADP); (2) zinc promotes binding between fibrinogen and integrin  $\alpha_{IIb}\beta_3$ ; (3) zinc enhances extracellular calcium influx into platelets; (4) zinc regulates the redox state of -SH moieties in the calcium channels, mediated by protein disulfide isomerase; and (5) zinc activates protein kinase C, which is regulated via a zinc redox mechanism. Interestingly, Kimura et al. [52] reported that zinc deficiency reduced granule substances without changing the numbers of megakaryocytes and platelets. Thus, zinc deficiency has a negative effect on platelet aggregation and prolongs bleeding times in adult males [12]. This result can be rescued with doses of 50 mg Zn/day [13], although doses of 30 mg/day were ineffective [17]. Importantly, Marx et al. [53] reported that 50 mg Zn/day caused a predisposition to increased platelet reactivity, which could cause a predisposition to increased coagulability without altering the platelet count.

A convenience sample (non-probability) was chosen in order to facilitate the study due to the high costs and labor involved and because the population was known to be homogeneous. Other limitations were a relative short duration of treatment, extensive dietary assessment, and logistical complexity. Additionally, the sample size of 50 schoolchildren was adequate for the conclusions obtained in this study because the sample size required would be  $n=15$ .

## Conclusions

Our results suggest that oral zinc supplementation may contribute to increased consumption of energy, fat, protein, and alkaline phosphatase by healthy children. Importantly, zinc increased HDL-c levels and decreased platelet count, both of which are of clinical significance. Furthermore, zinc has the potential to be used in dyslipidemias to elevate serum HDL-c levels [46]. Because there are

few reports of zinc supplementation in apparently healthy children, the influence of this intervention in this group must be studied in greater detail.

#### Acknowledgments

This study was supported by the National Council for Scientific and Technological Development (CNPq), grant no. 472832/2011-5. The authors do not have any relationships to disclose which would cause a conflict of interest.

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