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Plasma Zinc Concentration, Body Composition and Physical Activity in Obese Preschool Children

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Abstract Zinc (Zn) deficiency and obesity can be observed together in some developing countries. Zn deficiency may enhance fat deposition and decrease lean mass accrual, which in turn, appears to influence physical activity (PA), although this has not yet been evaluated in obese children. The objective of the study was to find out the association between measurements of plasma Zn and serum leptin, body composition, and PA in Chilean obese preschool children. Seventy-two 18- to 36-month-old obese children [weight-for-length/height *z* score (WHZ)>2.0 SD], belonging to low socioeconomic communities, participated in the study. Plasma Zn, serum leptin, weight, waist circumference, height, total body water (TBW) assessed by deuterium isotopic dilution technique and daily activity, measured by registering 48 h with an accelerometer, were evaluated. We found 82% of children with WHZ>3 SD. The geometric mean Zn intake was 6.2±2.5 mg/day. The mean plasma Zn was 91.8±11.4 μg/dL, with 10% of the children having levels <80 μg/dL. No correlation was found between plasma Zn concentrations and either weight, WHZ, or waist circumference. Serum leptin was lower in males than in females (2.9±2.8 vs 6.8±5.0 ng/mL, respectively; $p<0.001$). TBW was different between males and females (56.2±5.4 vs 52.8±4.3% body weight, respectively; $p=0.004$), but no significant association was found between TBW and plasma Zn. Moderate + intense PA, (as percentage of wake time), was greater in males than in females (6.3±3.1% vs 3.4±2.3%, respectively; $p<0.001$), but it was not significantly correlated to plasma Zn. In conclusion, plasma Zn was not associated with body composition as assessed by TBW, serum leptin, or with the magnitude of physical activity in Chilean overweight preschool children.

Keywords Zinc · Obesity · Body Composition · Deuterium · Total body water

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Introduction

Approximately 10% of school-aged children in the world are at risk of obesity and one-fourth of these are obese [1]. Demographic, socioeconomic, feeding patterns, and lifestyle changes may explain the rise in the prevalence of obesity in childhood and adolescence during the past three decades in Chile and in most of the world [2]. In Chile, urbanization and economical globalization have drastically modified the lifestyles of the population. Presently, 23% of children less than 6 years of age, as monitored by the National System of Health Services, are either overweight or obese. Similar rates can be observed in approximately 35% of preschoolers attending day-care centers [3].

Historically, obesity has been classically considered as a condition associated to abundance and/or wealth. Nevertheless, recent data shows that obesity rates are increasing in underprivileged populations, where furthermore, it coexists with some nutritional deficiencies [4]. Castillo-Duran et al., showed in 2001 that Chilean children living in urban slums had a higher risk of specific nutritional deficiencies, especially zinc deficiency, and in whom growth and psychomotor development were impaired [5], although this situation may have changed in more recent years, due to the country's continuous economic growth this past decade.

Some of the causes that might explain zinc deficiency in young children include short duration of exclusive breastfeeding, artificially feeding unfortified whole cow's milk, low consumption of animal products, and increased consumption of refined foods. A recent study showed that a vitamin- and mineral-fortified milk provided by the National Program of Complementary Feeding in Chile [6], might not be satisfying the requirements of zinc of 18-month-old children living in low socioeconomic communities [7].

It appears that zinc deficiency has an effect on body composition in experimental animals [8]. Furthermore, Golden et al. in the 1980s suggested that zinc had an effect on lean tissue deposition in children recovering form malnutrition [9].Nevertheless, it should be mentioned that in these studies, body composition was assessed mainly using skinfold techniques [10, 11]. Although in humans it has not been completely demonstrated, obese children with zinc deficiency might not only have a higher fat mass, but could also have lower rates of lean mass synthesis. Marreiro et al., found an association between obesity and lower plasma zinc concentrations in obese children [12].

Leptin is a hormone, which has been shown to be positively associated with body fat mass and recent energy intake [13]. Zinc appears to be associated with leptin, as one trial showed that lower leptin levels were observed during zinc deficiency, which subsequently increased following zinc repletion [14]. Furthermore, studies in infants have shown a positive relationship between them.

Zinc might not only be associated to obesity through its effect on body mass composition, but also due to its apparent effect on physical activity. One study in primates showed that those who experienced long-term zinc deficiency initiated before puberty were less active than those that were fed a zinc-sufficient diet during the premenarchial period [15]. Furthermore, community-based trials in Guatemala and India observed that toddlers who received zinc supplements were more active than those who received a placebo [16, 17].

The objective of the present study was to assess the association between plasma Zn and body composition and/or physical activity in overweight preschool children of low socioeconomic status.

Subjects and Methods

Children between 18 and 36 months of age were recruited between March 2004 and July 2006. Inclusion criteria into the study were: children of both genders, who had been born at term (≥37 weeks), with a birth weight >2,500 g, singleton, with no chronic diseases, who were at the time not attending day care centers and had been diagnosed as overweight or obese at La Pintana Health Care Center, in Santiago. Overweight [weight-for-length *z* score (WHZ) between 1.0 and 2.0 SD] and obesity (WHZ >2.0 *z* score) were assessed according to WHO standards [18]. Children with secondary causes of obesity (e.g., Prader Willi syndrome, hypothyroidism) were not considered for the study.

All children were weighed and their heights measured without clothing by the same field worker using standardized procedures [19]. Weight was measured using a mechanical scale with ±10 g precision (SECA Corp, Columbia, MD). Height was measured to ±0.1 cm precision with a stadiometer (SECA Corp, Columbia, MD). Height-for-age and WHZ were calculated according to the new WHO growth reference standards to assess the children's growth. Waist circumference was measured with plastic non-extensible tape at the umbilical level, and registered in centimeters, with one decimal.

Dietary recalls from the previous three days of the visit, which included a weekend day, were performed by a trained dietitian. Food composition tables from Chile [20] and USA [21] were used to calculate the children's energy, protein, iron, and zinc intakes.

Socioeconomic status was assessed using a modified Graffar scale, which has been adapted for Chile [22]. The scale includes data on family members, income, housing, marital status, parental schooling, number of beds, sewage and plumbing, social security status, and home appliances.

Body composition was evaluated using a deuterium dilution technique. A known oral dose of deuterium (0.2 g/kg as oxide, 99.9% purity; Cortec, Paris), diluted to 50% volume, was administered to all children after an overnight fast >8 h. Saliva samples (>2.5 mL) were collected previous to the deuterium administration and after 3 h, to comply with the Plateau protocol. Total body water (TBW) was calculated using the dilution principle, where deuterium concentrations and volumes administered and the deuterium concentrations and volumes in the saliva samples are known. Corrections were made for deuterium incorporation into labile protein pools and for water added to the doses. The deuterium enrichment was determined by comparing the deuterium content of saliva samples (0.4 mL), in triplicate, to that of a highly pure hydrogen standard (99.9%), using a Pt catalyzer and analysis done in a continuous flow mass spectrometer (Hydra, Europe Scientific, Crewe, UK).

Fomon coefficients (specific for sex and age) for the hydration of fat-free mass (FFM) were applied to values of TBW, to obtain FFM. Total body fat was obtained by difference of FFM of total body weight.

Physical activity was assessed using an activity monitor (Actiwatch 64 movement sensor, Minimeter, USA) [23]. The monitor sensor was attached to each child's chest into a specially designed tissue box for 48 h, and the saved information voided to a PC with a software program (Actiware©-Sleep, Mini Mitter Co.3.4).

An approach to the children's zinc nutritional status was obtained through the measurement of zinc in plasma. Fasting, whole blood samples (5 ml) were obtained from each subject between 9 and 10 A.M. For each of the samples, blood was immediately transferred to a trace element-free tube with heparin (100 U). All blood samples were centrifuged (2,500×*g*) for 10 min, within 2 h of drawing and subsequently stored in a −20°C freezer. Plasma zinc was measured by a dilution technique using atomic absorption spectrophotometer (Perkin Elmer, model 2280, Norwalk, CT). A venous blood sample was obtained, and serum was separated and kept frozen at −20°C until analysis of leptin concentrations was performed. Serum leptin was measured by RIA (Linco Research, Inc., St. Charles, MO, USA) as previously described and validated [24]. The intra- and inter-assay coefficients of variation were 2.5 and 3.7%, respectively.

The study was previously approved by the Ethics Committee of the Institute of Nutrition and Food Technology, Universidad de Chile, in accordance with the revised World Medical Association declaration of Helsinski. A written and informed consent was obtained from the mothers before admitting the children into the study.

Statistical Analyses

Statistical analyses were performed by SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL). Descriptive statistics and comparisons between genders are presented as means ± SD for normally distributed variables and as geometric means ± 1 SD for variables that were not normally distributed and had to be logarithmically transformed for their analysis. A two-factor analysis of variance (ANOVA) was used to compare differences between genders. All comparisons were done at the 5% level of significance.

Results

Table 1 shows the main characteristics of the children included in the study. The average age of the subjects was 27.3±6.2 months. The mean WHZ of the infants was 3.35±1.0. As expected, all of them but one were obese, with 82% of them having values >3 SD. Furthermore, the mean WHZ was not significantly different between females and males (3.43±0.9 vs 3.37±1.0) (Table 1). Similarly, the fat mass, as percentage of body weight, was also significantly higher in females than males (34.1±4.3% vs 31.3±5.3%, respectively; p=0.02). In addition, females had higher waist circumferences than males (58.1±5.4 vs

Table 1 Characteristics of Children Included in the Study (n=72)[a]

Characteristic	Male (n=43)	Female (n=29)	p value	Total (n=72)
Age (months)	26.2±5.8	29.1±6.5	0.05	27.3 ±6.2
Weight (kg)[b]	16.2±2.3	16.8±2.9	NS	16.4±2.5
Height (cm)	87.7±4.9	88.8±6.3	NS	88.1±5.5
Height-for-age z-score	−0.11±0.91	−0.05±1.16	NS	−0.08 ±1.0
Weight-for-height z-score[b]	3.37±1.0	3.43±0.9	NS	3.39±1.0
Weight-for-age z-score[b]	2.2±0.81	2.2±0.7	NS	2.3±0.9
Waist circumference (cm)[b]	55.7±4.8	58.1±5.4	0.05	56.6±5.2
Plasma Zn concentration (μg/dL)	89.9±11.9	94.6±10.5	0.09	91.8±11.5
Plasma leptin concentration (ng/ml)[b]	2.9±2.8	6.8±5.0	<0.001	4.1±4.3
Total body water (% body weight)	56.3±5.0	52.8±4.4	0.04	54.9±5.0
Fat mass (% body weight)	31.3±5.3	34.1±4.3	0.02	32.4±5.1
Moderate + intense physical activity (% of wake time)	4.8±0.3	2.3±0.4	<0.001	3.6±0.5

[a] Mean±SD, except where noted

[b] Geometric mean±1 SD presented; variables logarithmically transformed for analysis

Table 2 Nutrient Intakes of 18- to 36-month-old Chilean Overweight Infants (n=56)[a]

Nutrient	Intake
Energy (kcal/d)	1,650.6±387.5
Energy (kcal/kg/d)	101.7±27.0
Protein (g/d)	55.3±15.4
Protein from animal sources (as % of total protein)	69.2±12.0
Zinc (mg/d)[b]	6.2±2.5
Percent zinc from animal sources (%)	76.2±10.4
Calcium (mg/d)	843.3±286.8
Iron (mg/d)	10.2±3.9

[a] Mean±SD, except where noted;
[b] Geometric mean±1 SD

55.7±4.8), although the difference was not statistically significant (p=0.05) (Table 1). Data from the dietary recalls showed that the children had either normal or increased energy and protein intake, as compared to the recommendations for this age group. The mean energy and protein intake of the subjects was 1,650.6±388 kcal/day and 55.3±15.4 g/day, respectively, (Table 2). Approximately 70% of the protein intake came from animal sources. The geometric zinc intake (±SD) was 6.2±2.5 mg/day, and there was no difference in intake, by gender or between infants in the upper tercile of WHZ and those in the lower two terciles. In addition, only 5% of the infants had zinc intakes below current WHO recommendations for this age group (4.1 mg/day, assuming moderate bioavailability).

The mean plasma zinc concentration of the infants was 91.8±11.5 μg/dL, and only eight children (10%) had values <80 μg/dL (Table 1). Furthermore, there were no significant correlations between plasma zinc and either weight, WHZ, or waist circumference. There were also no statistical differences in plasma zinc concentrations by gender or between infants in the upper tercile for WHZ and those in the lower two terciles. On the other hand, plasma leptin concentrations, as geometric means, were significantly different between males and females (2.9±2.8 vs 6.8±5.0 ng/ml, respectively; p<0.001) (Table 1). Moreover, plasma leptin concentrations were greater in those infants in the upper tercile of WHZ compared to those in the lower two terciles (6.7±5.5 vs 3.2±2.7 ng/mL, respectively; p<0.001). On the other hand, no significant association was found between plasma zinc and serum leptin concentrations.

The total body water (TBW), as a percent of body weight, was on average 55% and was significantly greater in males than in females (56.3±5.0 vs 52.8±4.3%, respectively; p=0.04) (Table 1). TBW was significantly correlated to weight, WHZ, waist circumference, and leptin concentrations (p<0.001).

The mean percentage of moderate or intense physical activity, as a percent of time awake, was 3.6±0.5%, with males having significantly higher values than those of females (4.8±0.3 vs 2.3±0.4%, respectively; p<0.001) (Table 1). No significant statistical association was found between plasma Zn and the rate of moderate or intense physical activity.

Discussion

The objective of the present study was to assess the association between plasma zinc concentrations, as a measure of risk of zinc deficiency and body composition, as measured by total body water and physical activity in overweight infants of low socioeconomic Chilean communities. No significant relationship was found between plasma zinc concentrations and total body water in these children. Furthermore, no significant correlations was observed between plasma zinc and weight, weight-for-height and waist circumference, and no significant differences in body composition were found between children with marginal or adequate plasma zinc concentrations. Similarly, no differences in plasma zinc concentrations were observed between children in the upper tercile of WHZ and those in the lower two terciles. The lack of associations observed could have been due to the low percentage of infants who were at risk of zinc deficiency, as indicated by the fact that only 10% of them had marginal plasma zinc concentrations (<80 μg/dL).

Unlike 6 years ago, were it was observed that 55% of infants of similar ages from the same communities as those in the present study had plasma zinc concentrations <80 μg/dL [7], only 10% of the infants of the present study had marginal plasma zinc concentrations. In the period from 2001 to 2006, maternal schooling increased from 8.3 to 9.4 years; the rate of families in extreme poverty decreased from 41 to 25%, and the rate of families with social security increase from 58 to 81%. The increase of family incomes may explain, in part, the improvement in the quality of protein and zinc consumption (animal protein increased from 60 to 69% of total protein).

Furthermore, dietary history records from the previous study showed that close to 90% of the subjects had zinc intakes below WHO recommendations for these ages, whereas only 5% of the children in the present study had zinc-deficient intakes. Thus, it appears that the nutritional situation of these settings might have changed in the last six years, possibly due to the country's continuous economic growth this past decade.

The interaction between zinc and body composition continues to be a matter of debate. A few studies have shown that zinc may have an effect on body composition. Giugliano et al., demonstrated in experimental animals that Zn deficiency favored increased muscle turnover, with an increased muscle catabolism [25]. Furthermore, a correlation between Zn absorption and tissue Zn stores, mainly in muscle, has also been described in experimental animals [26]. Regarding human-based studies, Diaz-Gomez et al., showed a positive effect of zinc supplementation on total body water, as assessed by bioimpedance, in preterm infants, whose total body water was significantly higher than a control group after 3 and 6 months of supplementation [27]. Gibson et al., showed in 11-year old, New Zealand children, a correlation among body weight and hair zinc concentrations; both males and females with hair zinc concentrations >2.44 umol/g were heavier and had more body fat than those children with hair Zn below that concentration [28]. Furthermore, Kikafunda J et al., observed that zinc supplementation increased the middle upper arm circumference of preschool children and also led to a greater weight gain in these children compared to the placebo group [29]. It should be pointed out that the human-based studies mentioned above were conducted on non-overweight children. It should be pointed out that we are aware of only one previous study which has assessed the relationship between zinc and body composition in obese children. Marreiro et al. described that Brazilian obese children and adolescents appeared to have lower plasma zinc concentrations than their non-obese counterparts [12]. Nevertheless, these children were older than the infants included in the present study. In the present study, boys had higher total body water and less fat mass than girls, results that are similar to those found by Fomon et al. [30]. Important changes in body composition occur during the first 2 years of life. Studies in healthy children showed that fat mass decreases from 20.8 to 17.5% in males and from 21.8 to 18.5% in females, in the ages between 18 and 36 months [31]. Velasquez et al., assessed the nutritional status and body composition by deuterium dilution technique in Chilean preschool children and found out that in healthy children, TBW (as percent body weight) was 59.6±3.9% and fat mass was 22.2±4.7% [32]. The magnitude of TBW or fat mass found in our subjects is in agreement with these references and with the condition of obesity.

Serum leptin concentrations are intimately related to the amount of fat mass, which may explain the greater concentrations of serum leptin found in girls (more obese) compared to boys [33]. There are no reference data of serum leptin for children between 18 and 36 months of age. Experimental studies, both in mice and monkeys, have shown that zinc has an effect on the production of leptin, which could be a factor regarding regulation of energy metabolism [34, 35].

Our results show that boys were more physically active than girls. Given the difference in muscle mass between males and females, which is relatively small during preschool years, there is a different physiologic capacity for physical activity between genders [23]. Cultural aspects appear to play an important role in this difference, as they promote more sedentary activities for girls than for boys.

In conclusion, plasma zinc was not associated with body composition, as assessed by TBW, or with the magnitude of physical activity in Chilean overweight preschool children. Further observational or intervention studies are needed in obese children of this age range, to determine whether zinc has an effect on body composition.

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