Zinc and Iron Nutrition in Chilean Children Fed Fortified Milk Provided by the Complementary National Food Program

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OBJECTIVE: Chilean infants are at risk for isolated zinc and iron deficiencies because of a low consumption of animal products in low socioeconomic sectors. In 1999, the National Complementary Food Program of Chile manufactured a new milk (2 kg of powdered milk/mo) fortified with iron (Fe; 10 mg/L), zinc (Zn; 5 mg/L), and copper (0.5 mg/L) to be provided to infants until age 18 mo and to pregnant women. We analyzed the nutrition status of zinc and iron at age 18 mo in infants who consumed the fortified cow′s milk.

METHODS: Forty-two healthy male children with normal growth and from lower socioeconomic groups were studied. A nutrition survey was conducted; blood and hair samples for Zn in plasma and hair, hemoglobin, hematocrit, and serum ferritin were obtained.

RESULTS: Mean intakes were: energy, 106 ± 27 kcal · kg^{-1} · d^{-1}; protein, 3.8 ± 1.1 g · kg^{-1} · d^{-1}; Zn, 5.2 ± 1.9 g/d (0.98 mg Zn/MJ; 68% of World Health Organization recommendations); Fe, 11.2 ± 5.5 mg/d; and dietary fiber, 9.8 ± 3.9 g/d. Plasma Zn in 54.8% of children was no greater than 12.3 μM/L; 36% had hair Zn level no greater than 1.23 μM/g and 39% had serum ferritin levels no greater than 10 μg/dL (12% were anemic). Hair Zn was correlated to socioeconomic level (Spearman's rank correlation, $r = -0.53$; $P < 0.001$) and plasma Zn was correlated to the z weight/length ($r = 0.47$; $P < 0.05$), subscapular skinfold ($r = 0.46$; $P < 0.05$), and Zn intake ($r = 0.46$; $P < 0.05$).

CONCLUSIONS: The fortified powdered cow′s milk provided to infants until age 18 mo by the Complementary Food Program in Chile favorably affects the Fe status of these children, but possibly not the Zn nutrition; we suggest re-evaluation of the levels of Zn fortification. *Nutrition* 2004;20:177–180. ©Elsevier Inc. 2004

KEY WORDS: zinc, iron, body composition, infants, nutrition program

INTRODUCTION

As a developing country, Chile has evolved over recent decades toward improved socioeconomic conditions for its inhabitants. As a result, an epidemiologic transition stage is occurring. Prevalent diseases such as infant protein-energy malnutrition or acute diarrhea and low birth weight have decreased to rates closer to those of developed countries. However, isolated nutrient deficiencies, mainly those of iron, calcium, and zinc (Zn), persist in children from low socioeconomic groups.[1,2]

Iron deficiency is the most prevalent nutritional deficiency around the world, affecting children from poor communities, sometimes even in developed countries. Studies performed in Chile have shown that 20% to 40% of infants from low income groups present iron deficiency anemia.[3] A greater rate of infants present iron-deficient erythropoiesis and/or depleted iron stores. The adverse effects of iron deficiency during the first years of life go beyond the limited hemoglobin synthesis to more important deleterious effects such as decreased cellular immunity, behavior, and cognitive alterations.[4,5]

Zinc deficiency in humans was initially described by Prasad et al.[6] in 1961 in Iranian children. In Chile, this deficiency has been found in infants, preschool children, schoolchildren, and adolescents.[7,8] The greater prevalence in infants has been associated with low birth weight, early weaning, feeding with whole cow's milk–based formula, and complementary feeding low in meat products. Zinc nutrition has an important role in metabolism; Zn deficiency is associated mainly with deficient growth, sexual maturation, fertility, immunity, taste, and appetite.[9–11]

Cow′s milk is high in proteins, calcium, and phosphorus. In Chile it is common to recommend for children older than 6 mo a dilution of powdered whole cow′s milk to 8% and the addition of sugar and cereals. This dilution may increase the risk of micronutrient deficiencies (iron, zinc, and copper). Since 1999 the Complementary National Food Program (CNFP) in Chile has replaced the usual powdered whole cow′s milk by a similar milk with supplemented iron (10 mg/L), Zn (5 mg/L), copper (0.5 mg/L), and vitamin C (70 mg/L; Purita Fortificada, Santiago, Chile) to prevent these mineral deficiencies. The iron content with high bioavailability ensures an adequate amount of absorbed iron,[12] which may be effective in preventing iron-deficient anemia.[13] Also, studies of Zn bioavailability have shown increased zinc absorption with this milk.[14] However, studies evaluating the impact of Zn fortification on functional parameters in the target population (70% of Chilean

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TABLE I.

CHARACTERISTICS OF CHILDREN STUDIED ($N = 42$)*

Age (mo)	18 ± 0.5
Birth weight (g)	3559 ± 435
Weight (Kg)	11.5 ± 1.02
Length (cm)	81.5 ± 2.8
Socioeconomic status†	38.6 ± 6.6

* Values are mean ± standard deviation.
† According to a modification of Graffar's method.[15]

children belonging to low socioeconomic groups) are missing. This milk was previously evaluated for growth and macronutrient absorption in field studies.[12,13] The CNFP provides 2 kg/mo to all infants attending public primary health care centers until age 18 mo.

The objective of this study was to analyze the iron and Zn nutrition statuses of a sample of healthy 18-mo children from lower socioeconomic communities in Santiago, Chile.

MATERIALS AND METHODS

We studied 42 healthy male 18-mo-old children. Each subject attended a wellness baby clinic in a primary care center located in an urban slum of Santiago. All had been fed with fortified cow's milk for at least the previous 6 mo. We selected male rather than female children because of their apparent higher risk of showing clinical signs of Zn deficiency.[2]

There were several requirements to be a part of our study: low socioeconomic status according to a modification of Graffar's method,[15] age of 18 mo, birth weight of at least 2,500 g, normal weight according to growth standards of the National Center for Health Statistics and World Health Organization (NCHS-WHO), absence of congenital malformations or chronic diseases, not enrolled in a day-care center, and no vaccines during the previous 15 d.

The study was approved by the Ethics Committee at the Institute of Nutrition and Food Technology, University of Chile; mothers accepted the participation of their children by freely signing a written informed consent.

The children were evaluated according to the following parameters: weight, by using a digital SECA balance with a precision of 0.1 kg; length, as measured by a Harpenden stadiometer (Harpenden, Crymych, UK) with a precision of 0.1 cm; brachial circumference, as measured by a standard technique using measuring tape made of a non-extendable fiberglass; and tricipital and subscapular skinfold, by using a Lange caliper (Cambridge Scientific, Cambridge, MA, USA). Weight and length were used to calculate weight-for-age, length-for-age, and weight-for-length z scores according to NCHS-WHO standards.

A 24-h dietary history of infants was obtained from their mothers. To calculate daily nutrient intakes, a food-processor software was used, complemented by national food composition data.[16] The adequacy of energy and protein intakes was evaluated in relation to Food and Agriculture Organization, FAO/WHO/ United Nations University, UNU reference data; the adequacy of iron and Zn intakes were compared with WHO recommendations.[17]

A 4-mL blood sample was drawn from every child from an antecubital vein between 9 and 11 AM, after at least 8 h of fasting. The sample was inserted into a plastic tube containing 100 to 200 U of Zn-free heparin.

Hemoglobin and hematocrit in whole blood were analyzed by a hematologic counter (Celdyn 1700, Abbott Park, IL). Plasma ferritin was analyzed according to the method of the International Nutritional Anemia Consultative Group.[18] Anemia was defined as a hemoglobin level below 11 g/dL, and depleted iron stores was defined by a plasma ferritin level below 10 μg/dL.[12]

To analyze plasma Zn, the heparinized blood sample was centrifuged at 2500g; the plasma obtained was diluted to 1:5 with deionized water. The hair sample was cut from the occipital scalp by using stainless steel scissors, and only hair 2 cm close to the scalp was used. The samples were washed with neutral shampoo and then with deionized water; samples were dried at 60°C for 12 h; thereafter, they were digested with ultrapure nitric acid and hydrogen peroxide and diluted in distilled deionized water. The processed plasma and hair samples were analyzed for Zn in an atomic absorption spectrophotometer (model 2280, Perkin-Elmer, Norwalk, CT). We chose a lower cutoff point of 12.3 μMl/L (80 μg/dL) for normal plasma Zn and 1.23 μM/g (80 μg/g) for normal hair Zn based on our previous studies.[1]

The statistical analysis included means, standard deviations, and frequencies. Also analyzed were linear correlations (Pearson's or Spearman's rank order correlations) and chi-square results. An α error of 5% was considered statistically significant.

RESULTS

Thirty-six of the 42 children studied had normal nutrition status, three presented weight-for-age between + 1 and +2 z scores, and three presented weight-for-age between −1 and −2 z scores, but all had normal weight-for-length. Mean length was 81.5 ± 2.8 cm, with 11 children (26.2%) with length-for-age between −1 and −2 z scores (NCHS-WHO standards). With regard to infants' families, 70% of mothers had at least 8 y of schooling, and 17% had at least 12 y. Mean family income was US $187/mo (US $37/member); 46% had incomes below the Chilean minimal salary (US $176 during 2001). According to the modified Graffar method, the families belonged to low income groups (the higher scores correspond to the poorer families; Table I).

The dietary surveys showed energy intakes between 64.5 and 163 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1} (38% of the children had below the recommendations for age), 28% of energy intake from fats, a protein

TABLE II.

DIETARY SURVEY (24-H HISTORY) OF CHILDREN STUDIED ($N = 42$)

	Energy (Kcal $\cdot$ kg^{-1} $\cdot$ d^{-1})	Protein (g $\cdot$ kg^{-1} $\cdot$ d^{-1})	Fat (g/d)	Carbohydrate (g/d)	Ca (mg/d)	Dietary fiber (g/d)	Fe (mg/d)	Zn (mg/d)
Mean	106	3.8	40.2	170.7	830	9.8	11.2	5.2
±SD	27	1.1	12.8	42.5	326	3.9	5.5	1.9
Confidence limits	64.5, 163	2.5, 6.8	25.1, 64.8	95.1, 268	281, 1848	2.8, 21.7	3.3, 25.7	2.5, 11.5

Ca, calcium; Fe, iron; SD, standard deviation; Zn, zinc

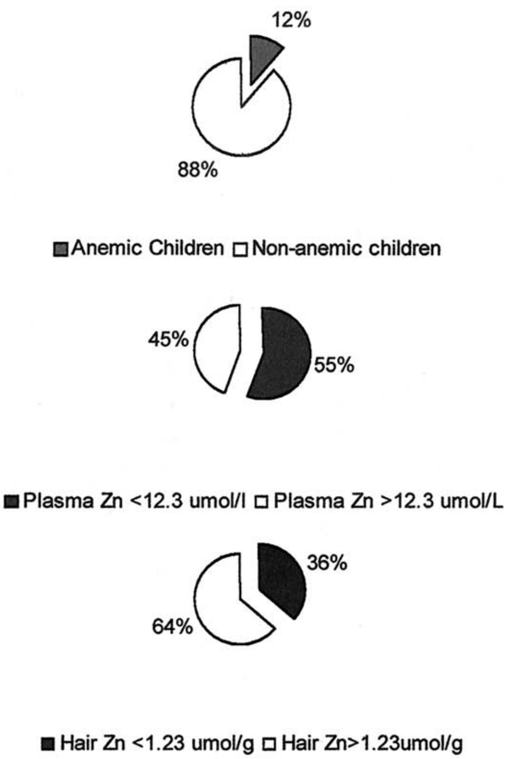

FIG. 1. Percentage of children with anemia: hemoglobin < 11 g/dL, plasma Zn ≤ 12.3 μM/L (80 mg/dL), and hair Zn ≤ 1.23 μM/g (80 mg/g). Four of five children with anemia presented serum ferritin levels below 10 μg/dL. Zn, zinc.

intake of 2.5 to 6.8 $g \cdot kg^{-1} \cdot d^{-1}$ (all children had more than the recommendations), and a ratio of 60% from animal sources.

Zinc intake ranged from 2.6 to 11.5 mg/d; only four children presented an intake above the WHO recommendations for Zn.[19] The mean ratio of Zn to energy was 0.98 mg Zn/MJ (68.4% of WHO recommendations).

Iron intake ranged between 3.3 and 25.7 mg/d, with 18 children (42.3%) presenting levels higher than recommended. Calcium intake was between 281 and 1848 mg/d, and dietary fiber was between 2.8 and 21.7 g/d (Table II).

In measurements of iron nutrition, 12% of children had ferropenic anemia, and 39% had serum ferritin levels below 10 μg/dL, suggesting depleted iron stores (Figure 1). With respect to Zn status, 23 children (54.8%) presented plasma Zn levels no higher than 12.3 μM/L and 14 children (36%) had hair Zn concentrations no greater than 1.23 μM/g (Table III).

A linear correlation was found between plasma Zn and weight-for-length ratio ($r = 0.47$; $P < 0.05$), between plasma Zn and Zn intake ($r = 0.46$; $P < 0.05$), between plasma Zn and subscapular skinfold ($r = 0.46$; $P < 0.05$), and between hair Zn and socioeconomic level (Spearman's rank order, $r = - 0.53$; $P < 0.001$).

DISCUSSION

Different studies have demonstrated the effectiveness of fortified milk formulas in the prevention of iron deficiency in infants.[12,13] However, in the case of Zn, the studies have been mostly geared toward studying the nutrition status of Zn.[20] In Chile, since 1999, infants to age 18 mo have been fed 2 kg/mo of powdered whole milk fortified with iron, Zn, copper, and vitamin C. In the present study we noted that these infants' diets contained, for the most part, the suggested caloric and protein requirements (WHO/UNU recommendations: 102 $kcal \cdot kg^{-1} \cdot d^{-1}$ and 1.2 $g \cdot kg^{-1} \cdot d^{-1}$, respectively) consistent with those needed for normal growth. Normal growth requires the consumption of micronutrients for the creation of new tissue and for use in a large number of enzymatic reactions. Golden et al.[21,22] studied malnourished infants supplemented with Zn and found a decrease in the energy cost for new tissue, suggesting that this effect was mediated by a relative increase in lean tissue stores. Subsequent studies in children and animals have reported similar findings.[23–25]

The consumption of this milk seems to be able to prevent iron-deficient anemia because the percentage of anemia was only 12% as compared with other studies carried out before the use of this milk, in which the prevalence of anemia was 30% to 40% in the same group.[3,12,13] This is of great importance because 39% of the infants showed decreased iron deposits (ferritin <10 μg/dL), and anemia may be associated with altered health including psychomotor deficits.[4] Moreover, results by Walter et al. with similar infants[5] indicated that, despite the treatment of anemia with iron supplements between the ages of 12 and 15 mo, previously anemic infants presented lower scores on intelligence tests at age 5 y compared with controls with a normal iron diet.

With iron, in contrast, we did not observe such preventive effects in the case of milk fortified with Zn. Despite fortification with 5 mg/L, 2 mg more than the normal content in the unfortified powdered cow's milk, we found a high rate of infants with Zn concentrations no greater than 12.3 μM/L (54.8%) in their plasma and Zn concentrations no greater than 1.23 μM/g (36%) in their hair samples. The observation that this milk prevents iron deficits but not zinc deficits could be explained by the different magnitudes of fortification with these micronutrients as compared with their requirements. With 10 mg/L of iron fortification, this implies that, with the consumption of 600 to 700 mL of milk daily (customary for infants at this age), they receive 6 to 7 mg/d of iron, which is equal to 65% to 70% of the recommended amounts for this age.[17] With the same milk intake and with the Zn content of 5 mg/L, the children would receive 3.0 to 3.5 mg Zn/d, which is only 40 to 44% of the WHO recommendations for this age.[17] If we analyze the results of the nutrition survey, we see that meat provides only 0.8 mg of iron and 0.7 mg of Zn per day. Thus, most of the

TABLE III.

IRON AND ZINC STATUS ASSESSMENTS OF CHILDREN STUDIED ($N = 42$)

	Hematocrit (%)	Hb (g/dL)	Ferritin (μg/dL)	Plasma Zn (μM/L)	Hair Zn (μM/g)
Mean ± SD	36 ± 2.3	12.1 ± 1	9.6 (4.4–21.3)*	12.7 ± 1.9	1.4 ± 0.5
Range	30.1–40.1	10.7–14.0		8.4–18.4	0.5–3

Hb, hemoglobin; SD, standard deviation; Zn, zinc
* Geometric mean and range of ±1 SD

micronutrients for this age group come from the fortified milk that is being provided. This begs the question of whether the milk is being fortified with enough Zn and whether it is necessary to increase the levels. The concentrations of Zn in plasma and hair samples in addition to the dietary records used in this study suggesting the risk of Zn deficiency are frequently used to analyze Zn nutrition but are not very sensitive, especially in the case of marginal deficiencies. The effects of Zn supplements on specific parameters, such as in immunity and growth rates, are necessary to corroborate results and to define the correct amount of Zn to be included in the fortification of milk.

The negative correlation observed between socioeconomic status and the concentration of Zn in the hair samples is concordant with the fact that a large part of ingested Zn and its availability originate from animal products, which are more expensive. In addition, poorer families have a higher risk of infections such as respiratory infections and gastrointestinal infections, which are important causes of the loss of ingested Zn.

In summary, these results are important because this is the first study conducted in children who received fortified milk from the CNFP in Chile. With these results we can infer that this milk has favorably influenced the nutrition status of iron in children but seems to have produced a lesser impact on the nutrition status of Zn.

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