

Zinc absorption from a micronutrient-fortified dried cow's milk used in the Chilean National Complementary Food Program

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Abstract

The objective was to compare the absorption of zinc from a micronutrient-fortified dried cow's milk, which is part of the Chilean National Complementary Food Program, with that from a standard nonfortified milk. Fortification included the addition of iron, zinc, copper, and ascorbic acid to provide total concentrations (in 100 g of powder) of 10, 5, 0.5, and 70 mg, respectively. Seven adults with normal plasma zinc concentrations ($16.0 \pm 0.9 \mu\text{mol/L}$) were studied. Fractional absorption of zinc was determined from single test meals using a stable isotope-based methodology. Mean (\pm SD) fractional absorption of zinc of the test meal containing nonfortified milk was 0.29 ± 0.09 compared with 0.30 ± 0.09 of the test meal with the fortified milk ($P =$ not significant). Absolute zinc absorptions from the test meals were 0.43 ± 0.14 vs 0.57 ± 0.16 mg, respectively ($P = .02$). Micronutrient fortification of dried cow's milk, including zinc, was associated with increased total zinc absorption.

Keywords: Zinc; Micronutrients; Milk; Food fortification; Humans

1. Introduction

Long-term nutrition and health policies, in addition to economic improvements, have been key factors in determining a reduction of nutritional deficiencies in Chile. Infant malnutrition

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in children younger than 6 years is less than 1%. Children with a weight-for-age score between -1 and -2 SD is only 7%. Stunting is virtually absent in the infant population [1]. A major nutritional program is the National Complementary Food Program (NCFP). Currently, this program distributes 18 000 tons of foods to 872 000 beneficiaries. The total cost is around US\$38 million per year. This program distributes 26% fat powdered milk directed to children younger than 18 months and all pregnant women, cereal-milk mix for those between the 18 months and 5 years, and rice for children and pregnant mothers at risk for malnutrition [2]. Despite all efforts, some micronutrient deficiencies, including zinc, are still present in specific groups. For instance, zinc deficiency has been identified in infants [3], children [4], short-stature adolescents [5], and pregnant adolescents [6]. At the end of 1999 to early 2000, the regular 26% fat powdered milk was replaced by a micronutrient (Fe, Zn, Cu, ascorbic acid)–fortified version. There were no data on zinc absorption from this fortified milk. In addition, it was of interest to evaluate some potential interference of zinc absorption by iron [7]. This study assessed zinc absorption of the fortified and the nonfortified milks by using a zinc stable isotope–based methodology.

2. Methods and materials

2.1. Subjects

Seven apparently healthy adults, 5 men and 2 women (mean age, 41.9 ± 10.9 years; body mass index, 23.9 ± 3.5 kg/m²), were studied. Each participant was fully informed about the characteristics of the study, and a written informed consent was signed. The study was approved by the Ethical Committee of the Department of Nutrition, Faculty of Medicine, University of Chile.

2.2. Study design

Fractional absorption of zinc (FAZ) from single test meals based on the fortified and nonfortified dried cow's milk preparations was measured in apparently healthy adult volunteers using zinc stable isotope extrinsic labels and a dual isotope ratio technique [8]. Zinc absorption was determined from single test meals consumed in lieu of breakfast on 2 consecutive days consisting of 330 mL reconstituted milk powder (fortified on day 1 and nonfortified on day 2) and labeled with ⁶⁷Zn and ⁶⁸Zn, respectively, and 2 pieces of white toast plus butter. Thus, each subject was his/her own control. A replicate of this procedure was conducted 1 month later. In this occasion, however, nonfortified milk was provided on day 1 and fortified milk on day 2. No significant differences were observed in both determinations. Zinc absorption was considered as the average between both determinations.

2.3. Micronutrient fortified and nonfortified milk

The nonfortified milk corresponded to regular dried 26% fat cow's milk, distributed countrywide as part of the NCFP under the brand name Leche Purita. The fortified milk Leche Purita Fortificada corresponds to the same product plus the addition of selected nutrients. The fortified milk contains (in 100 g powder): iron, 10 mg (as ferrous sulfate); zinc, 5 mg (as acetate); copper, 0.5 mg (as cupric sulfate); and ascorbic acid, 70 mg.

2.4. Preparation and administration of zinc stable isotope solutions

Accurately weighed quantities of preparations of zinc oxide enriched with ^{67}Zn , ^{68}Zn , or ^{70}Zn (Oak Ridge National Laboratory, Oak Ridge, Tenn) were dissolved in 0.5 mol/L H_2SO_4 to prepare a stock solution. For preparation of orally administered doses of ^{67}Zn and ^{68}Zn , the stock solutions were diluted with triply deionized water and titrated to pH 5.0 with metal-free ammonium hydroxide. For the intravenously administered ^{70}Zn , the pH of the stock solution was adjusted to 6.0 with ammonium hydroxide and the stock solution was diluted with sterile isotonic sodium chloride to a zinc concentration of 1.5 mmol/L. Oral and intravenous solutions were filtered through a 0.2- μm filter. The zinc concentrations of these solutions were measured by atomic absorption spectrophotometry with mass correction factors applied [9]. The intravenous doses were tested for pyrogens and sterility immediately before use.

2.5. Isotope administration

Preparations of zinc stable isotopes (^{67}Zn and ^{68}Zn) were administered orally as extrinsic tracers, according to the protocol described previously. Accurately weighed quantities of isotope solutions (approximately 0.8 mg) were combined with fortified or nonfortified milk and allowed to equilibrate for 12 hours. The subjects drank 330 mL of milk and the 2 pieces of white toast plus butter. The container with the labeled milk was rinsed with deionized water twice, and these rinses were also ingested. Test meals were provided at 8:30 AM. During the following 3 hours, the subjects had to refrain from taking anything by mouth but deionized water.

An accurately weighed quantity (approximately 0.5 mg) of a third tracer (^{70}Zn) was administered intravenously before the oral administration of labeled test meal of day 1. Administration was with a 10-mL syringe, 3-way stopcock, and scalp vein needle into a superficial vein in the forearm over a 5- to 10-minute interval. The syringe was flushed twice with isotonic sodium chloride solution using the 3-way stopcock.

2.6. Sample collections and analyses

Timed 30 to 50 mL urine samples were collected daily in acid-washed Nalgene bottles from days 4 to 9 after administration of the first tracer. Urine sample procedures and zinc stable isotope analyses have been provided in detail elsewhere [10]. Percentages of ^{67}Zn , ^{68}Zn , and ^{70}Zn enrichments were determined by inductively coupled plasma mass spectrometer in the Pediatric Nutrition Laboratory, University of Colorado Health Sciences Center, Denver. With the exception of ICP-MS determinations, all procedures were conducted at the Department of Nutrition, Faculty of Medicine, University of Chile.

2.7. Test meal analyses

Zinc concentrations in both fortified and nonfortified milk, as well as duplicates of the test meals used to evaluate zinc absorption, were analyzed by atomic absorption spectrophotometry with a Perkin-Elmer AAnalyst-100 spectrophotometer (Perkin-Elmer Co, Norwalk, Conn).

2.8. Plasma zinc

Before the infusion of the zinc stable isotope dose administered intravenously, 3 mL of blood was obtained to analyze plasma zinc concentration subsequently determined by atomic absorption spectrophotometry according to the method of Smith et al [11].

2.9. Dietary data

Dietary information was obtained from two 24-hour dietary recalls. Energy and nutrient intakes were calculated with the FP2 software (ESHA Research, Salem, Ore) by using a database that contained locally generated nutrient composition data as well as information from the literature [12].

2.10. Data analysis

Data were analyzed by using the SPSS 10.0 statistical software (SPSS Inc, Chicago, Ill). Zinc absorption from the 2 test meals was compared by the paired *t* test. A probability less than .05 was considered as significant.

3. Results and discussion

The NCFP was implemented in Chile in 1937. The program reaches at least 95% of the target population. Several adjustments according to the country's epidemiological evolving reality have taken place in recent years. Based on information related to the prevalence of iron deficiency in the age group between 0.5 and 2 years, and pregnant women [13,14], plus local studies suggesting also the presence of zinc deficiency [3-6], the regular 26% fat dried cow's milk was replaced by a micronutrient fortified version. The aim of this study was to provide information about the program's potential benefits in terms of zinc nutriture.

Mean (\pm SD) plasma zinc of the individuals studied was $16.0 \pm 0.9 \mu\text{mol/L}$. Mean dietary intakes of energy, protein, iron, copper, and zinc intakes were 8.43 ± 1.94 MJ (1992 ± 463 kcal), 70.3 ± 13.9 g, 14.2 ± 4.7 mg, 1.3 ± 0.4 mg, and 8.3 ± 1.6 mg, respectively.

Actual mean zinc concentration of the nonfortified milk was 3.06 ± 0.3 mg/100 g. The fortified milk had 4.62 ± 0.2 mg/100 g. Fractional zinc absorption of the test meal containing fortified cow's milk was not significantly different to the test meal with nonfortified milk. Total absorbed zinc from the test meals with fortified milk was greater than the nonfortified version (Table 1).

Table 1
Fractional and absolute zinc absorptions from test meals with nonfortified and micronutrient fortified milk

	Nonfortified milk	Fortified milk	<i>P</i>
Test meal Zn (mg) ^a	1.44 ± 0.06	1.92 ± 0.04	<.001
FAZ	0.29 ± 0.09	0.30 ± 0.09	.86
Absorbed Zn (mg)	0.43 ± 0.14	0.57 ± 0.16	.02

Values are expressed as mean \pm SD.

^a Does not consider the amount of zinc supplied by the labels.

Issues regarding mineral bioavailability are quite relevant in food fortification programs. To address this issue in terms of zinc, the analysis should focus on the potential effects on the target groups. Nevertheless, any conclusions drawn from these data in relation to the infant have to be qualified by the recognition that extrapolation of results for a single test meal may not be the same as those derived from actual measurement of zinc absorption from all meals for 1 day. These conclusions also depend on extrapolation from adult data. Recent kinetic results indicate that efficiency of zinc absorption in infants is lower than adults (KMH, unpublished data, 2005). With these caveats in mind, however, it is useful to calculate the quantity of zinc that would be absorbed by infants from this fortified milk.

Infants receive 2 kg milk powder per month, which will provide 92.4 mg Zn or 3.1 mg Zn per day. With an FAZ of 0.30, 0.93 mg Zn per day would be absorbed from this milk compared with 0.59 mg Zn per day from the nonfortified milk. The recent calculations of the Food and Nutrition Board of the Institute of Medicine give a figure for physiological requirement of 0.84 mg Zn per day [15]. Hence, this relatively modest fortification program could possibly make a crucial difference to the adequacy of zinc intake of older infants and toddlers.

Regarding pregnant women, the only available data on dietary zinc in this group in Chile are from pregnant adolescents who had a mean zinc intake of 7.4 ± 2.3 mg/d [6]. This is lower than the current recommendations of the Food and Nutrition Board of the Institute of Medicine [15]. Pregnant women receive, as part of the NCFP, 1 kg of the dried milk powder per month, which will contain about 46 mg zinc, that is, an average daily addition of 1.53 mg dietary zinc. With an FAZ of 0.30, this would provide additional 0.46 mg zinc per day of absorbed zinc. The corresponding increase in absorbed zinc from the nonfortified milk would have been 0.29 mg Zn per day. The increment in absorption of zinc required during the third trimester of pregnancy to meet the accumulation by the conceptus is approximately 0.75 mg. Thus, if the poor pregnant Chilean woman is dependent on this free milk program to meet these extra needs, the current fortification program will help but not alone be sufficient. However, the gap can be narrowed to some extent by increase in zinc absorption in late pregnancy [16].

In conclusion, micronutrient fortification of dried cow's milk, including zinc, was associated with increased total zinc absorption from a single test meal. This fortification program could help to meet the increase in physiological requirements of zinc for pregnant women and make a crucial difference to the adequacy of zinc intake of older infants and toddlers.

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References

- [1] Uauy R, Atalah E, Kain J. The nutritional transition: new nutritional influences on child growth. In: Martorell R, Haschke F, editors. Nutrition and growth. Philadelphia: Williams and Wilkins; 2001. p. 305-28.

- [2] Atalah E. Programa nacional de alimentación complementaria (PNAC). In: Rebolledo A, Bustos P, editors. Manual de alimentación y nutrición humana. Santiago: Departamento de Nutrición, Facultad de Medicina, Universidad de Chile; 2003. p. 105-9.
- [3] Castillo-Durán C, Rodríguez A, Venegas GV, Alvarez P, Icaza G. Zinc supplementation and growth of infants born small for gestational age. *J Pediatr* 1995;127:206-11.
- [4] Ruz M, Castillo C, Lara X, Codoceo J, Rebolledo A, Atalah E. A 14-month zinc supplementation trial in apparently healthy Chilean preschool children. *Am J Clin Nutr* 1997;66:1406-13.
- [5] Castillo-Durán C, García HR, Venegas P, Panteon E, Torrealba I, Concha N, et al. Zinc supplementation increases growth velocity of male children and adolescents with short stature. *Acta Paediatr* 1994;83:833-7.
- [6] Castillo-Durán C, Marín VB, Alcázar LS, Iturralde H, Ruz M. Controlled trial of zinc supplementation in Chilean pregnant adolescents. *Nutr Res* 2001;21:715-24.
- [7] Solomons NW, Ruz M. Zinc and iron interactions: concepts and perspectives in the developing world. *Nutr Res* 1997;17:177-85.
- [8] Friel JK, Naake VL, Miller JV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. *Am J Clin Nutr* 1992;55:473-7.
- [9] Peirce PL, Hambidge KM, Goss CH, Miller LV, Fennessey PV. Fast atom bombardment mass spectrometry for the determination of zinc stable isotopes in biological samples. *Anal Chem* 1987;59:2034-7.
- [10] Hambidge KM, Huffer JW, Raboy V, Grunwald GK, Westcott JL, Sian L, et al. Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids. *Am J Clin Nutr* 2004;79:1053-9.
- [11] Smith Jr JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clin Chem* 1979;25:1487-91.
- [12] Schmidt-Hebbel H, Pennacchiotti I. Tabla de composición química de alimentos Chilenos. Santiago: Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile; 1985.
- [13] Ruz M. Trace element intake and nutriture in Latin America. In: Wahlqvist ML, Truswell AS, Smith R, Nestel PJ, editors. Nutritional in a sustainable environment. London: Smith-Gordon and Co; 1994. p. 296-300.
- [14] Hertrampf E, Olivares M, Letelier A, Castillo C. Situación de la nutrición de hierro en la embarazada adolescente al inicio de la gestación. *Rev Med Chil* 1994;122:1372-7.
- [15] Food and Nutrition Board. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academy Press; 2001.
- [16] King JC. Effect of reproduction on the bioavailability of calcium, zinc and selenium. *J Nutr* 2001;131:1355S-8S.