Published in final edited form as:
Food Nutr Bull. 2015 June ; 36(2): 111–119. doi:10.1177/0379572115586400.

Prediction of Serum Zinc Levels in Mexican Children at 2 Years of Age Using a Food Frequency Questionnaire and Different Zinc Bioavailability Criteria

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Abstract

Background—The 2006 Mexican National Health and Nutrition Survey documented a prevalence of zinc deficiency of almost 30% in children aged one to two years old.

Objective—We sought to validate a Food Frequency Questionnaire (FFQ) for quantifying dietary bioavailable zinc intake in two-year old Mexican children accounting for phytic acid intake and using serum zinc as a reference.

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The authors declare no competing financial interests

Funding disclosure
This work was supported by the National Institutes of Health/National Institute of Environmental Health Sciences (R01-ES007821 and P20-ES018171), the U.S. Environmental Protection Agency (RD83480001), and Consejo Nacional De Ciencia y Tecnología (CONACYT 41912-M). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH/NIEHS or USEPA.

Methods—This cross-sectional study was nested within a longitudinal birth cohort of 333 young children in Mexico City. Non-fasting serum zinc concentration was measured and dietary zinc intake was calculated on the basis of a semi-quantitative FFQ administered to their mothers. The relationship between dietary zinc intake and serum zinc was assessed using linear regression, adjusting for phytic acid intake, and analyzed according to two distinct international criteria to estimate bioavailable zinc. Models were stratified by zinc deficiency status.

Results—Dietary zinc, adjusted for phytic acid intake, explained the greatest proportion of the variance of serum zinc. For each mg of dietary zinc intake, serum zinc increased on average by 0.95 μg/dL (0.15 μmol/L) (p=0.06). When stratified by zinc status, this increase was 0.74 μg/dL (p=0.12) for each milligram of zinc consumed among children with adequate serum zinc (N=276) whereas among those children with zinc deficiency (N=57), serum zinc increased by only 0.11 μg/dL (p=0.82).

Conclusion—A semi-quantitative FFQ can be used for predicting serum zinc in relation to dietary intake in young children, particularly among those who are zinc-replete, and when phytic acid/phytate intake is considered. Future studies should be conducted accounting for both zinc status and dietary zinc inhibitors to further elucidate and validate these findings.

Keywords

Zinc Status; Bioavailable zinc; Phytic Acid; Children; Food Frequency Questionnaire

Introduction

In Mexico, the 1999 National Nutrition Survey reported a 34% prevalence of low serum zinc levels among children under two years of age [1]. Subsequently, the 2006 National Health and Nutrition Survey (*ENSANUT-2006* by its abbreviation in Spanish) found that up to 11.4% of children under five years had zinc intakes below the Estimated Average Requirement (EAR) [2] and 29.2% of children aged one to two years had zinc deficiency [3]. Zinc deficiency was associated with an estimated 400,000 deaths worldwide in 2004 among children under five years [4].

Zinc, an essential nutrient which participates in numerous enzyme systems [5,6] and contributes to the regulation of cell proliferation, plays a key role as a necessary nutrient for growth, brain development and immunity [7,8]. Moderate zinc deficiency in pre-school aged children is common in developing countries [8,9] and can delay linear growth, inhibit the immune response, suppress appetite and reduce the capacity to detect flavors [10–12].

The United Nations Food and Agriculture Organization and the World Health Organization (WHO) have classified diets into three categories depending on whether zinc bioavailability is low, moderate or high. These categories take into account: 1) absorption enhancers, 2) absorption inhibitors and 3) the phytic acid:zinc ratio in the diet [13]. Primary zinc deficiency is common in young children [14] as zinc concentrations in breast milk reach >3 mg/L at the beginning of lactation but drop to <1 mg/L throughout the first six months of life [15]. Infants of this age also depend on other foods in order to satisfy their physiological requirements; thus it is important to provide them with quality complementary feeding.

Foods with low zinc and high phytate levels will lower zinc absorption to less than two-thirds of intake and are likely to culminate in moderate zinc deficiency [14].

Evaluating risk of zinc deficiency in children requires analysis of their dietary histories (breast and complementary feeding patterns) and gastrointestinal pathology reports [16]. Despite the widely documented lack of adequate biomarkers for measuring zinc, serum zinc has been found to be the most suitable for evaluating the status in infant and young children[17] and is recommended by both WHO and the International Zinc Consultative Group (IZiNCG) [18,19].

Given the public health importance of zinc in Mexican children and considering that food frequency questionnaires (FFQ) offer a low-cost, easily applicable tool for estimating usual zinc intake, this study was aimed at validating an FFQ to estimate the dietary zinc intake of young children using serum zinc as a reference biomarker of zinc concentrations. The association between serum zinc and dietary zinc parameters was analyzed to account for bioavailable zinc adjusting for phytic acid intake and compared to recognized international criteria.

Methods

Study Population

This cross-sectional study was nested in the second birth cohort (1999–2001) of the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) Project. We evaluated 333 two-year-old children affiliated with the Mexican Social Security Institute in Mexico City, who attended study visits at the National Institute of Perinatology Isidro Espinosa de los Reyes (*INPer* by its abbreviation in Spanish), between 2001 and 2003 [20,21]. During morning visits, non-fasting venous blood samples were drawn from children, and mothers answered questions regarding their child’s usual dietary intake in the three previous months. This study was evaluated and approved by the Research, Ethics and Biosafety Committees of the National Institute of Public Health of Mexico (*INSP* by its abbreviation in Spanish) and participating institutions. Mothers of the cohort-enrolled children were apprised of the study procedures and objectives, and signed an informed consent prior to participation.

Assessment of Dietary Zinc

ELEMENT used a semi-quantitative FFQ to estimate dietary intake in children, which was originally validated by Hernández-Ávila et al. [22] for adult females of low-medium socioeconomic level residing in Mexico City using the Willett methodology [23].

Administered by trained, standardized personnel, the FFQ included 116 foods grouped into ten categories (dairy products, fruit, vegetables, legumes, cereals, sweets, beverages, fats, snacks, eggs and meats). The list of foods was built on the items that proved most representative of local consumption under the 1983 Dietary Survey of the Mexican National Institute of Nutrition [24]. Additionally, the questionnaire included ten frequency values ranging from never to six or more times a day.

The zinc content of each item on the list (for a standard portion size: one customary unit, cup, slice, piece, etc.) was obtained mainly from food composition tables supplied by two sources [25]: 1) the United States Department of Agriculture (USDA) and 2) the Mexican National Institute of Nutrition and Medical Sciences Salvador Zubirán (*INNCMNSZ* by its abbreviation in Spanish) [26]. Data were compiled by specialized personnel at INSP.

Average daily dietary nutrient (e.g., zinc, phytic acid) intakes were calculated with a computer program developed at INSP. Pre-determined food portion sizes were applied to mothers' FFQ responses. The following values were used to estimate the equivalent frequencies consumed per day: 0 = never, 0.016 = less than once a month, 0.08 = two-three times a month, 0.14 = once a week, 0.43 = two-four times a week, 0.8 = five-six times a week, 1 = once a day, 2.5 = two-three times a day, 4.5 = four-five times a day and 6 = six or more times a day. Next, these frequencies were multiplied by the nutrient content in each food item and summed over all food sources of the nutrient.

While several validation studies recommend the incorporation of data on multivitamin intake [27], only three children in our study (<1%) had taken zinc-containing vitamins and the doses were minimal. Therefore, we did not include a multivitamin-use variable in our analyses.

Estimation of Bioavailable Zinc

Phytate:zinc molar ratios were calculated using the IZiNCG equation [19], which takes into account the molecular weight of both components ((phytate/660)/(zinc/65.4)). Bioavailable zinc in the children's diets was then estimated using two international criteria:

a. *Bioavailable Zinc (mg/d) according to WHO criteria*: 50% zinc absorption where phytate:zinc molar ratios fall under 5; 30% absorption where they range from 5 to 15, and 15% absorption where they exceed 15[28].

b. *Bioavailable Zinc (mg/d) according to IZiNCG criteria*: 31% zinc absorption in children where phytate:zinc molar ratios range from 5 to18 (corresponding to mixed diets), and 23% absorption where they exceed 18 (corresponding to vegetarian and whole-grain diets) [18].

Serum Zinc Levels

Whole-blood samples were centrifuged and serum was isolated at the *INPer* laboratory, where zinc concentrations were determined by flame atomic absorption spectrometry (FAAS), the technique deemed most suitable for this procedure [29]. Serum zinc values were used as the criterion measure, and children were classified as having zinc deficiency with values below 65 μg/dl [30].

Covariates

Maternal characteristics, such as mother's age and education at delivery, and infant weight and length at birth were recorded. Weight and length were also measured at 24 months. WHO Anthro (version 3.2.2, January 2011, World Health Organization, Geneva, Switzerland) software was used to evaluate the nutritional status of participants.

Statistical Analysis

Descriptive statistics (means and standard deviations) were computed for zinc and phytate levels and prevalence of low zinc intake (below EAR %: <2.5 mg/d) and serum zinc deficiency were calculated.

To analyze FFQ capacity for predicting serum zinc levels (dependent variable), separate linear regression models were formulated with the following independent variables:

1. Dietary zinc intake (mg/day)
2. Dietary zinc intake (mg/day) adjusted for phytic acid intake (mg/day)
3. Phytate:zinc molar ratio
4. Zinc bioavailability (mg/day) according to WHO criteria
5. Zinc bioavailability (mg/day) according to IZiNCG criteria

Additionally, models were stratified by zinc status, to assess whether the FFQ predicted the serum-dietary zinc relationship differently among children who were zinc replete compared to those who were zinc deficient.

All statistical analyses were performed using STATA 11.0 (StataCorp LP, College Station, Texas USA, Copyright 1985–2009).

Results

Table 1 shows a list of the principal sources of zinc (>0.5 mg/100 g) served as a basis for calculating the daily intakes from FFQ contained in the following food groups: dairy products, eggs and meats, legumes, and cereals.

Table 2 describes the relevant demographic characteristics and dietary parameters of the sample population. Mean (±SD) zinc intake was 8.0 (±2.3) mg/d, with only one child below the EAR of 2.5 mg/d[31]. Phytic acid intake was 545.2 (±190.1) mg/d with an average phytate:zinc molar ratio of 7 (interquartile range: 5.4 – 8.4). Using IZiNCG criteria, only 31% of zinc consumed was absorbed and the average bioavailable zinc was 2.5 (±0.7) mg/d. By the WHO criteria, 97% of the children had zinc absorption of 50% (phytate:zinc molar ratio <5). Variance in mean bioavailable zinc estimated by the two criteria was statistically different (p=0.02). Average serum zinc was 79.6 (±16.1) μg/dL (12.2 ± 2.5 μmol/L), and this was higher, though not significantly, in females (80.3 ± 16.3 μg/dL, 12.3 ± 2.5 μmol/L) than in males (78.9 ± 16.3 mg/dL, 12.1 ± 2.5 μmol/L) (p=0.41). Serum zinc deficiency was present in 17% of children with no difference by sex (females 15.5% and males 18.7%, p=0.43).

Table 3 shows the results of linear regression models used to compare various methods of dietary zinc estimation as predictors of serum zinc. Total dietary zinc (mg/d), as estimated directly from the FFQ, was not a statistically significant predictor of serum zinc in the full sample or when stratified by zinc status. Adjusting for phytic acid intake, for every additional milligram of zinc consumed, serum zinc increased on average by 0.95 μg/dL (0.15 μmol/L) (p=0.06). Among children without zinc deficiency (N=276), this increase was 0.74

μg/dL (p=0.12) for each milligram of zinc consumed, whereas among those children with zinc deficiency (N=57), serum zinc increased by only 0.11 μg/dL (p=0.82).

For each unit increase in the phytate:zinc molar ratio, serum zinc decreased on average by 0.69 μg/dL (0.11 μmol/L) (p=0.13) indicating that for the same amount of zinc intake, higher phytic acid intake may decrease zinc bioavailability. In the models stratified by zinc status, this negative relationship was present in those children without zinc deficiency ($\beta = -0.74$, p=0.1), and not in those children with zinc deficiency (β=0.04, p=0.9).

Under the WHO and IZiNCG criteria, serum zinc increased by an average of 1.40 (−0.26, 3.08) and 0.68 (−1.77, 3.14) μg/dL (0.21 (−0.04, 0.47) and 0.10 (−0.27, 0.48) μmol/L), respectively, with each additional mg of bioavailable zinc in the diet.

Discussion

This is the first study to validate the use of a FFQ for estimating zinc consumption using a serum zinc reference biomarker in young Mexican children and provides the first comparison of zinc bioavailability criteria used by international organizations. In addition, this study offers evidence that phytic acid intake should be accounted for when estimating dietary zinc intake. This is important for populations consuming non-Westernized diets and in developing countries where diets tend to be more plant-based as well as for plant-based or vegetarian eaters in developed countries who tend to eat more phytic acid than omnivores [32].

As this study includes a sample of children from Mexico City, the prevalence of zinc deficiency is lower (17%) than the previously reported at national level (34%) because it is expected that urban children would have better nutrition status than children in rural areas of Mexico. Also, even though the mean dietary zinc intake of the sample (8 mg/d) is 1 mg higher than the upper tolerable (UL) intake level of 7 mg/d, this did not reflect an over-consumption of zinc because the high intake of phytic acid in Mexican population inhibits the absorption of the majority of the zinc consumed [3].

Earlier studies have validated the use of diverse dietary assessment instruments for dietary zinc evaluation [33] and, of these, the semi-quantitative FFQ is the most commonly used in epidemiological studies to evaluate dietary intake, especially long-term intake (>7 days). Few studies have demonstrated the impact of zinc intake, absorption-related factors, and diet quality on serum concentrations [34,35]. This may be due to the fact that few studies in children have characterized usual zinc intake accurately.

The association observed between the FFQ and the reference method in our population demonstrates that dietary zinc adjusted for phytic acid consumption is the most suitable predictor of serum zinc in young children. Zinc intake analyses that do not account for intake of phytic acid, the principal zinc absorption inhibitor, may lead to less accurate estimates of dietary zinc intake especially in populations with high phytic acid intake. This finding is consistent with a previous study that designed an FFQ to measure zinc intake and recognized that the lack of information on phytic acid intake posed an important drawback [33].

The observed associations become more evident when analyzed according to zinc status. Higher zinc intake increased serum zinc only 15% as much, on average, in the zinc deficient children compared to the zinc-replete subgroup. Due to the limited sample size of the study, we cannot rule out chance for these findings. In addition, apparent zinc deficiency (i.e. low zinc concentrations without necessarily low total-body amounts) may be triggered by factors other than diet, namely: infection [36], inflammatory response [37], or other conditions that alter its metabolism [12]. In this respect, one of the shortfalls of our study includes the lack of available information on the children's recent illnesses, immunological conditions or inflammatory biomarkers, such as C-reactive protein measurements. However, we had information on lymphocyte count, so we included this information as a proxy for inflammation in a sensitivity analysis and the results were similar (data not shown).

Comparing different methods for estimation of zinc bioavailability in our study demonstrated that: 1) average intake varies considerably according to the method used and 2) none of the methods predict serum zinc levels adequately. This may arise from the fact that their cutoff values involve wide calculation ranges and their designs are aimed at adults, not children.

We are aware that FFQs have limitations in calculating nutrient intakes, since the respondents' recall plays a major role in their responses [27,38]. In addition, dietary assessment and evaluation in young children is complicated given that dietary habits change rapidly in the early years of life, parents share their feeding responsibility with other adult caregivers and may not be completely aware of children's diet, and children do not necessarily consume all of the foods given to them [39]. Our study is not completely free of three well-recognized sources of FFQ-related measurement error: 1) the food portions in a semi-quantitative FFQ are pre-determined and may not have been recognized or remembered consistently and therefore may have distorted our estimated daily zinc intakes; 2) the instrument was originally designed for epidemiological studies on chronic diseases to evaluate the diets of adult females, a population group which consumes more varied foods than our sample subjects; and 3) the FFQ does not measure certain types of foods consumed by children, such as a broadly distributed multi-fortified milk provided to low income families (e.g., Liconsa fortified milk) and other processed infant foods that tend to be zinc-enriched. However, structured interviewer-administered questionnaires were conducted by trained personnel to assist with portion size determination, recall, and capture of common foods not queried on standard form by prompting for additional information in open-ended questions at the end of the survey.

Based on our findings, a semi-quantitative FFQ can be used for predicting serum zinc in relation to dietary intake in young children, particularly among those who are zinc-replete, and when phytic acid/phytate intake is considered. Future studies should be conducted accounting for both zinc status and dietary zinc inhibitors to further elucidate and validate these findings.

Acknowledgments

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Table 1

Foods with zinc content ≥ 0.5 mg/100 g included in the semi-quantitative FFQ

Food	Portion size	Zinc mg/100g
DAIRY PRODUCTS		
Whole milk	1 glass (240 ml)	0.5 – <1.0
Cheese	1 slice (30 g)	2.0 – <3.0
Yogurt	1 typical cup of yogurt (150 g)	0.5 – <1.0
EGGS MEATS		
Egg	1 complete	0.5 – <1.0
Pork	½ medium-size steak (45 g)	2.0 – <3.0
Beef	½ medium-size steak (45 g)	≥ 3.0
Sausage or ham	1 sausage or 1 slice of ham (30 g)	1.0 – <2.0
Beef or chicken liver	1 piece (90 g)	≥ 3.0
Fresh fish	½ medium-size fillet (45 g)	0.5 – <1.0
Canned tuna	¼ can or 40 g	1.0 – <2.0
LEGUMES		
Beans	a) ½ plate or ½ cup from the pot (50 g)	0.5 – <1.0
CEREALS AND GRAINS		
Rice	½ cup or ½ plate (50 g)	0.5 – <1.0
White bread	1 loaf or ½ roll (35 g)	0.5 – <1.0
Whole wheat bread	1 loaf or ½ roll (35 g)	1.0 – <2.0
Sweet bread	1 piece (70 g)	0.5 – <1.0
Boxed cereal	1 cup dry (30 g)	≥ 3.0
Tortilla and tortilla products	1 piece	0.5 – <1.0

Table 2

Characteristics and dietary parameters estimated by a semi-quantitative FFQ among Mexican children at two years of age (N=333)

Variable	Mean (or %)	SD
Sex (male)	49%	
Birthweight (kg)	3.1	0.4
Birth length (cm)	50	1.8
Weight (kg)	11.9	1.5
Height (cm)	86.3	2.9
Breastfed for 12 months (yes)	27%	
Serum zinc (μg/dl)	79.6	16.1
Zinc deficiency (<65 mg/dl)	17%	
Dietary zinc intake (mg/d)	8.0	2.3
Phytic acid intake (mg/d)	545.2	190.1
Phytate:zinc molar ratio	6.9	2.0
Bioavailable zinc according to WHO (mg/d)[a]	2.6	1.0
Bioavailable zinc according to IZiNCG (mg/d)[b]	2.5	0.7
Energy (kcal/d)	1655.2	437.7

[a]World Health Organization (WHO) criterion [28]

[b]International Zinc Nutrition Consultative Group (IZiNCG) criterion [18]

Table 3

Association between serum zinc and dietary zinc parameters, stratified by zinc status, among Mexican children at two years of age

Dietary parameter	All participants (N=333)		Zinc replete (N=276)		Zinc deficient (N=57)	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Zn (mg/d)	0.22 (−0.54, 0.98)	0.58	0.03 (−0.69, 0.77)	0.92	0.15 (−0.55, 0.86)	0.66
Zn (mg/d) adjusted for phytic acid intake (mg/d)	0.95 (−0.029, 1.94)	0.06	0.74 (−0.20, 1.68)	0.12	0.11 (−0.88, 1.10)	0.82
Phytate:zinc molar ratio	−0.69 (−1.58, 0.19)	0.13	−0.74 (−1.64, 0.15)	0.1	0.04 (−0.61, 0.70)	0.9
Bioavailable zinc according to WHO[a]	1.4 (−0.26, 3.08)	0.1	1.14 (−0.42, 2.72)	0.15	0.54 (−1.25, 2.35)	0.54
Bioavailable zinc according to IZiNCG[b]	0.68 (−1.77, 3.14)	0.58	0.11 (−2.25, 2.49)	0.92	0.49 (−1.80, 2.79)	0.66

[a] World Health Organization (WHO) criterion [28]

[b] International Zinc Nutrition Consultative Group (IZiNCG) criterion [18]