Biol Trace Elem Res (2014) 161:38–47
DOI 10.1007/s12011-014-0090-9

Dietary and Non-dietary Factors Associated with Serum Zinc in Indian Women

Catherine A. Herbst · Kavitha C. Menon · Elaine L. Ferguson · Christine D. Thomson · Karl Bailey · Andrew R. Gray · Sanjay Zodpey · Abhay Saraf · Prabir Kumar Das · Sheila A. Skeaff

Received: 11 May 2014 / Accepted: 22 July 2014 / Published online: 2 August 2014

Abstract Women in low-income settings, common in India, are at risk of inadequate zinc intake due to poor diet quality and low consumption of flesh foods rich in zinc. The aims of this study were to assess the prevalence of zinc status of non-pregnant rural and tribal women living in central India and to identify dietary and non-dietary factors associated with the biochemical zinc status of these women. Rural and tribal non-pregnant women 18–30 years of age were selected using proportion to population sampling near Nagpur, Maharashtra, India. Sociodemographic, biochemical (serum zinc), clinical, and dietary data (1-day interactive 24-h recall) were collected. The mean age of women (n=109; rural=52; tribal=56) was 23.2 years and mean BMI was 17.9 kg/m^2. The majority of the participants identified as being non-vegetarian (72 %). The mean±SD serum zinc concentration was 10.8±1.6 μmol/L, and 52 % of participants had a low serum zinc concentration according to the International Zinc Nutrition Consultative Group (IZiNCG). The median (first and third quartile) energy, zinc intake, and phytate/zinc molar ratio was 5.4 (4.2, 6.7) MJ/day, 5.3 (3.8, 7.0) mg/day, and 26 (22, 28), respectively. Zinc intakes were well below IZiNCG recommendations for dietary zinc of 9 mg/day for non-pregnant women aged 14–18 years and 7 mg/day for non-pregnant women aged ≥19 years. Using linear regression analysis to identify non-dietary and dietary factors associated with serum zinc, a significant association was only found for current lactation (p=0.012) and energy intake (p<0.001). Diets low in energy with poor bioavailability of dietary zinc are likely to be the primary cause of the high proportion of Indian women with zinc deficiency.

Keywords Zinc · Phytate · Women · Deficiency · India

C. A. Herbst · C. D. Thomson · K. Bailey · S. A. Skeaff (✉)
Department of Human Nutrition, University of Otago, PO Box 56, Dunedin 9054, New Zealand
e-mail: sheila.skeaff@otago.ac.nz

K. C. Menon
Lifestyle Science Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertram, 13200 Kepala Batas, Pulau Pinang, Malaysia

E. L. Ferguson
London School of Hygiene and Tropical Medicine, London, UK

A. R. Gray
Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand

S. Zodpey
Public Health Foundation of India, New Delhi, India

A. Saraf · P. K. Das
Health and Family Welfare Training Institute, Nagpur, India

Introduction

Zinc has a myriad of functions in the human body and is needed for optimal immune and reproductive function, growth, appetite, and general metabolism [1]. The International Zinc Nutrition Consultative Group (IZiNCG) states that the likely primary cause of zinc deficiency is inadequate dietary intake of absorbable zinc [1]. Women in low-income settings are at risk of inadequate zinc intake due to poor diet quality and low consumption of flesh foods rich in zinc [2, 3]. This is true for women in India where vegetarianism is practiced because of local custom, religion, or economic constraints [4]. Demographic, health, and nutrition surveys of representative samples of rural and tribal populations in India have not directly measured zinc intake [5] but describe diets low in flesh foods and high in cereals in non-pregnant non-lactating women ≥18 years [6–10]. Cereals are rich in phytate, a potent inhibitor of zinc absorption.

To our knowledge, only two studies have reported on the dietary zinc intake of non-pregnant Indian women. They report a mean zinc intake in rural women of 4.7–6.0 mg/day and in tribal women of 3.1 mg/day [11, 12]. These intakes are below the IZiNCG estimated average requirement (EAR) of 7 mg/day for women ≥19 years consuming an unrefined cereal-based diet [1]. The lack of data on dietary zinc status is likely to be due to missing food composition database values for zinc, particularly in locally consumed foods [13]. There is also a shortage of well-designed studies examining serum zinc, another marker of zinc status in a population. The aims of this study were to assess the prevalence of the zinc status of non-pregnant rural and tribal women living in central India and to identify dietary and non-dietary factors associated with biochemical zinc status in these women.

Methods

Research Design and Study Population

This cross-sectional study was part of a wider study that examined the micronutrient status of rural and tribal women of reproductive age in Ramtek Block, Nagpur District, Maharashtra State, India [14]. Ramtek Block is located northeast of the city of Nagpur, located in central India, and is comprised of both rural and tribal communities. A rural population is defined as one with no more than 400 people/km^2 with the majority of males employed in agricultural work, while tribal people are considered to be a separate ethnic group that is socially underdeveloped. Although the tribal communities in Ramtek Block retained many aspects of their distinct culture, they were also primarily involved in agriculture. In April and June 2007, women aged 18–30 years were recruited using a stratified cluster sampling technique. Detailed descriptions of the sampling and exclusion criteria are outlined in Menon et al. [14]. In order to have 80 % power to detect a difference of 30 % in the prevalence of zinc deficiency (defined as serum zinc concentration <10.7 μmol/L for a fasting and <10.1 μmol/L for a non-fasting morning sample in non-pregnant women [1]) between rural and tribal women, assuming the worst case of an overall prevalence of 50 %, using a two-sided test at the 0.05 level, 50 women were needed in each strata (i.e., rural or tribal) for a final sample size of 100 women. Informed consent was obtained after verbal and written explanation of the study was given to each participant and her family. The Ethics Committee of the Health and Family Welfare Training Centre, Nagpur, India, and the University of Otago Ethics Committee approved the study.

Detailed sociodemographic, anthropometric, and biochemical data collection and analysis methods have been outlined in Menon et al. [14]. In brief, data collection occurred over a 2-day period. On day 1, participants were visited in their homes where a trained research assistant administered a pre-tested sociodemographic questionnaire; participants were instructed about the fasting blood sample collection to occur the following day. On day 2, participants attended their local health center, had their weight and height measured according to standardized methods for the determination of BMI (kg/m^2), and had fasting blood samples collected by trained medical staff. Participants then returned to their homes, and after consuming a meal, an interactive 24-h dietary recall for the previous day was conducted. Of the 109 participants who took part in the study, a 24-h dietary recall was not obtained from six participants due to logistic constraints. The present study examined the dietary zinc intake of 103 women and outlines in detail the methods of dietary data collection, coding and analysis, as well as biochemical zinc assessment.

Dietary Assessment

Dietary intake was assessed using an interactive, multiple pass, 24-h dietary recall method [15]. Dietary data were collected by two trained research assistants, one who was from Nagpur and fluent in the local language and the other fluent in Hindi and English. In the first pass, each participant was asked to recall all the foods and beverages consumed in the previous 24-h period, starting from early in the morning and progressing sequentially throughout the day. During the second pass, the time of food consumption was recorded and the amount of each food item consumed was estimated using household ingredients obtained from the woman's home, wherever possible. Participants were asked to place the amount of food on a plate, or an estimated volume of fluid, into a bowl. If the participant did not have the food item available at the time of the visit, the closest substitute food item was used instead. Foods were weighed using digital electronic scales (Salter Electronic, Salter Housewares Ltd., Tonbridge, UK, ±1 g) and recorded. In the third and final pass, the interviewer read all the recorded food items and beverages back to the participants who were then asked if any food item had been forgotten or left out so that these items could be added, if necessary.

To estimate the ingredients of composite meals, the recipe of the composite meal was recorded in the second pass and each raw ingredient within the recipe was weighed wherever possible. The participant was then asked to estimate the amount of the composite meal made for the entire household. The participant poured into a large vessel the volume of water representative of the amount of the total cooked ingredients of the composite meal prepared for the entire household, which was then weighed and recorded. To estimate the quantity of the composite meal that the participant had consumed, the participant poured into the bowl the volume of water representative of the amount of the composite meal they had consumed in the previous 24-h period; this was weighed and recorded. The proportion of the raw ingredients consumed for an individual was calculated as a proportion of the water equivalent weight for the amount of food consumed by the water equivalent weight for total amount of food prepared for the entire household times the weight of each raw ingredient that was cooked for the entire household. Raw ingredients within the composite meals were coded.

Dietary recalls were coded to calculate the weight in grams of every food item as a raw ingredient. Some 24-h dietary recalls had food items with missing weights. For these foods, a median weight was used, which was calculated from 24-h recalls collected from other participants. For example, the median weight of a potato was estimated by calculating the median weight of all potatoes consumed from the 24-h dietary recalls of participants who consumed potatoes. The missing weights for some food items could not be calculated in this way as either only one person consumed these food items or no weight was recorded on any of the 24-h dietary recalls that contained these foods. For these foods, the food weight of one adult serving was sourced from Malaysian, FAO, or UK food tables [16–18].

Some of the individual ingredients within the composite meals were also missing. These ingredients were estimated using the "calculated average recipe" (termed "average weight equivalent conversion factors for raw ingredients" by Gibson and Ferguson [15]). In brief, to create the calculated average recipe, each raw ingredient weight and the weight of the total cooked ingredients in the composite meal of interest were compiled from participants who consumed the recipe of interest (as long as there were more than three participants). Each raw ingredient recorded within these composite meals was divided by the weight of the total cooked ingredients to calculate the proportion of that ingredient within the recipe. This ingredient proportion was then calculated for every recipe of the composite meal of interest and a mean proportion determined to create the calculated average recipe. The mean proportion of each ingredient was then multiplied by the amount a participant consumed of the composite meal of interest on any given dietary recall to estimate the individual ingredient weight consumed by a participant. A calculated average recipe was obtained and used for 12 different composite dishes.

In cases where less than three participants consumed a composite meal, it was not possible to formulate a calculated average recipe. Instead, the "cooked average recipe" was used. Cooked average recipes for 21 rural and 23 tribal commonly consumed composite dishes were prepared by local women in both the rural (n=3) and tribal (n=2) settings, and all ingredients weighed and the final cooked dish were recorded. The proportion of each ingredient in the cooked average recipe was calculated in the same way as the "calculated average recipe." If a composite meal of interest did not have a recipe and had no "calculated average recipe" or "cooked average recipe," then the most appropriate recipe was sourced from a generic recipe and nutritive book created by the Indian Central Food Technological Research Institute [19]. Lastly, if none of the above methods could be used to ascertain the recipe for a given composite meal of interest, the most appropriate recipe (n=3) was sourced from the Internet.

Nutrient Analysis

Food intakes were converted into nutrient intakes including zinc and phytate using the Indian food composition database [13], which was scanned into Microsoft Excel and checked for accuracy, with macros created to calculate nutrient values. If a food item was not available or a nutrient was missing from the Indian composition database, then the following composition tables were used in a hierarchal order: Malaysian food composition table [16], Infoods food composition database [17], British food composition database [18], and the USDA (US Department of Agriculture) food composition table [20]. Any food items that were obscure and could not be sourced in these food composition databases were sourced from the following food composition databases in hierarchal order: British food composition supplement called "Immigrant Foods" [21], British food composition supplement called "Cereals and cereal products" [22], British food composition supplement called "Fish and fish products" [23], International Minilist of foods [24], and the New Zealand food composition tables [25].

As nearly all food items in the completed food composition database were inserted as raw values, the USDA retention factors [26] were used to adjust for zinc loss during cooking. To determine the most appropriate retention factor, the closest food and cooking method was selected. The only exception was processed takeaway foods as these were inserted into the food composition database as whole cooked values and the retention factors for these foods were already taken into consideration. A total of 101 food items were consumed by the participants in this study and included in the database.

Biochemical Assessment

Trained medical staff administered a questionnaire prior to blood sample collection to ascertain the participant's fasting status and, if not fasting, the recent food consumption, and the presence of acute and/or chronic illness. A fasting peripheral venous blood sample was taken with the participant in a lying or sitting position; the time of collection was recorded. Blood collection and processing was standardized following the strict protocols described by IZiNCG [1]. Blood samples for serum zinc analysis were collected into trace-element-free (TEF) vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA) and into plain vacutainers for C-reactive protein (CRP) analysis (Becton Dickinson, Franklin Lakes, NJ, USA). Separation occurred as soon as possible after collection (average time=2.25 h; 50 % within 2 h) at a laboratory in a rural hospital in Ramtek using TEF techniques. Serum was aliquoted in TEF polythene vials and stored at −20 °C until analysis. Serum zinc was analyzed by standard flame atomic absorption spectroscopy in the Department of Paediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi. Precision of the zinc assay was assessed using a pooled serum sample and certified reference material (SERO AS, Asker, Norway), and the inter-assay coefficient of variation (CV) was 8.3 %. The manufacturer and certified control values were within the specified ranges for serum zinc. The prevalence of low biochemical zinc status was defined using the IZiNCG reference value for a fasting morning sample in non-pregnant women of 10.7 μmol/L and for a non-fasting morning sample in non-pregnant women of 10.1 μmol/L [1]. CRP was measured by turbidimetry using CRP Turbiltex reagents (Agappe Diagnostics, Mumbai, India) at the National Reference Pathological Laboratory in Nagpur. The reference limit for CRP was >6 mg/L as specified by the manufacturer and was used as a marker of infection.

Statistical Analysis

Stata 9.2 (Stata Corporation, College Station, TX, USA) was used for data analysis, and the survey command was used to account for the effects of stratification and clustering. The survey weight was obtained by dividing the sample population by the total population for rural and tribal women. Rural women were weighted by a factor of 130.6 (i.e., 6,922/53), and tribal women were weighted by a factor of 239.2 (i.e., 13,393/56). For all distributions, histograms were visually examined to assess data normality and means and SDs or medians and quartiles were calculated as appropriate. Geometric means and SDS were investigated where a log-normal distribution was plausible and used where this was sufficiently close to the median.

Unadjusted linear regression was performed to determine if differences were present between rural and tribal women with respect to age, BMI, and total household income. All other socioeconomic categorical indices were summarized as percentages, and Pearson's chi-squared test was used to assess differences in proportion between rural and tribal women.

Nutrient intakes per day are presented as median (first and third quartiles) for clarity, as protein, calcium, iron, and phytate were log transformed to remove skew within strata. Differences in daily intake of selected nutrients between rural and tribal women were examined using *t* tests. The proportion of those women below the estimated average requirement (EAR) was compared using Pearson's chi-squared test.

Possible dietary and non-dietary factors thought to be associated with serum zinc were examined by unadjusted regression analysis after participants with elevated CRP (>6 mg/L; n=6) and those who used oral contraceptives were removed (n=1). Continuous non-dietary factors examined included age, BMI, total household income, years of education, and number of household members. Categorical non-dietary factors examined included being vegetarian, currently lactating, whether the blood sample was obtained from women who had fasted overnight or not, currently married, current parity, and living in a tribal community. Dietary factors included that were thought to have an effect on serum zinc were energy, protein, calcium, iron, zinc, phytate, and the phytate/zinc molar ratio [27]. Only variables that were significant at $p<0.20$ in the unadjusted analysis were included in the adjusted models for dietary and non-dietary factors (two separate models). A final model included those variables with $p<0.20$ in the adjusted non-dietary or adjusted dietary model. All residuals were plotted as histograms and scatterplots against fitted values to ensure the assumptions of the linear regressions models were met. The significance level for all statistical tests was two-sided $p<0.05$.

Results

The sociodemographic characteristics and health information of all participants (n=109) are summarized in Table 1. The overall mean±SD age of the participants was 23.2±3.6 years. Mean annual household income equated to approximately US$946 per annum [14]. The majority of the participants identified as being non-vegetarian (72 %) where vegetarianism was defined as being one of the following: vegan, lacto-vegetarian, lacto-ovo vegetarian, or ovo-vegetarian. At the time of collection, 22 % of the participants were currently breastfeeding and 51 % had given birth. The mean BMI of participants was 17.9 kg/m^2, and 63 % of participants had a BMI below the healthy range (i.e., <18.5 kg/m^2) [28] with 47 % of these underweight women having a BMI <16 kg/m^2, which is classified by the Food and Agriculture Organisation of the United Nations (FAO) as chronic energy deficiency grade III or "severe underweight" [29, 30]. Only two participants, both tribal women, were above the optimum BMI range, with one classified as overweight and the other as obese [28]. No statistically significant differences between rural and tribal women were found for any of the sociodemographic characteristics or BMI categories.

The mean±SD serum zinc concentration for all participants was 10.8±1.6 μmol/L, and there was no significant difference found between rural (11.3±2.1 μmol/L) and tribal (10.5±1.3 μmol/L) women (p=0.123) (Table 2). According to IZiNCG, if the population of interest has >20 % prevalence with a low serum zinc, it is identified as having elevated risk of zinc deficiency [1]. In the present study, 52 % of participants

Table 1 Characteristics of women of reproductive age from Ramtek Block, central India

	Rural (n=53)	Tribal (n=56)	All participants (n=109)
Years of age	23.2 (3.3)	23.2 (3.7)	23.2 (3.6)
Average annual income per household in Indian rupees (geometric mean)[a]	49,928	41,279	44,043
BMI (kg/m^2)	17.5 (2.5)	18.1 (3.1)	17.9 (2.9)
BMI (%)			
≥18.5 kg/m^2	32	39	37
<18.5 kg/m^2	68	61	63
Underweight[b] % (BMI, kg/m^2)			
Grade I, 17.0–18.49	33	27	29
Grade II, 16.0–16.99	19	27	24
Grade III, <16	47	47	47
Education (%)			
School ≤8 years	28	25	26
School >8 years	72	75	74
Number of members in household (%)			
≤5 members	60	57	58
>5 members	40	43	42
Food habits (%)			
Vegetarian	25	30	28
Non-vegetarian	75	70	72
Current marital status (%)			
Married	74	48	57
Not married	26	52	43
Current lactation status (%)			
Lactating[c]	26	20	22
Non-lactating	74	80	78
Parity (%)			
Nulliparous	40	54	49
Biparous	11	9	10
Multiparous	49	37	41

Table modified from Menon et al. [14]. Values stated as mean (standard deviation) unless stated otherwise. No significant difference between rural and tribal women observed for any parameters as tested by regression analysis for continuous variables and chi-squared test for association

[a] Conversion to US$: US$1=46.5 Indian rupees

[b] Underweight, BMI<18.5 kg/m^2 (n=70)

[c] Lactating (n=25)

had a low serum zinc concentration (39 % of rural women and 58 % of tribal women; p=0.054).

Median (first and third quartiles) energy, macronutrients, calcium, iron, zinc, phytate, and phytate/zinc molar ratio are presented in Table 3, along with the percent of women with an intake <EAR using values published by the US Institute of Medicine [31]. Median (first and third quartiles) dietary zinc intake for participants was 5.3 (3.8, 7.0) mg/day (Table 3). IZiNCG suggest an EAR for zinc intake in non-pregnant women who consume unrefined cereal-based diets of 9 mg/day for non-pregnant women aged 14–18 years and 7 mg/day for non-pregnant women aged ≥19 years [1]. The observed median in the present study is below the EAR for both age groups. As stated by IZiNCG, a population with more than 25 % of people below the EAR is considered to be at elevated risk of inadequate dietary zinc intakes [1]. In the present study, 78 % of participants had dietary zinc intakes lower than the EAR; 72 % of rural and 82 % of tribal women (p=0.420). IZiNCG estimates that an unrefined cereal-based diet has a phytate/zinc molar ratio of >18 [1]. The median (first and third quartiles) phytate/zinc molar ratio in the present study was 26 (22, 28). No statistically significant differences were found

Table 2 Mean (SD) zinc concentration and percent of women with low serum zinc

	Rural (n=49)	Tribal (n=53)	All participants[a, b] (n=102)
Serum zinc (μmol/L)	11.3 (2.1)	10.5 (1.3)	10.8 (1.6)
% low serum zinc	39	58	52

Table modified from Menon et al. [14]. Excludes participants with CRP >6 mg/L (n=6) and oral contraception use (n=1)

[a] No significant difference between rural and tribal women for either serum zinc or percent low serum zinc

[b] Cutoffs used were <10.7 μmol/L for fasted (n=90) and <10.1 μmol/L for non-fasted participants (n=12)

Table 3 Median (first and third quartiles) of energy, macronutrients, calcium, iron, zinc, phytate, and phytate/zinc molar ratio per day of women of reproductive age from central India and proportion below EAR

Nutrient per day	Rural (n=53)	Tribal (n=50)	All participants (n=103)	% of participants below EAR[a]
Energy (MJ)	5.3 (4.2, 6.7)	5.4 (4.2, 6.3)	5.4 (4.2, 6.7)	93
Protein (g)[a]	31 (23, 42)	32 (24, 41)	32 (23, 42)	40
Carbohydrate (g)	249 (182, 285)	236 (191, 278)	237 (187, 285)	–
Fat (g)	26 (15, 36)	23 (16, 34)	25 (15, 36)	–
Calcium (mg)	220 (135, 312)	243 (129, 331)	226 (131, 327)	99
Iron (mg)[b]	8.3 (6.1, 13.5)	9.1 (6.0, 12.7)	9.0 (6.0, 12.9)	44
Zinc (mg)[b]	5.6 (3.8, 7.2)	5.3 (3.8, 6.8)	5.3 (3.8, 7.0)	71
Phytate (mg)	1,261 (1,006, 1,994)	1,278 (999, 1,741)	1,264 (999, 1,795)	–
[Phytate]:[zinc] molar ratio	25 (22, 28)	26 (22, 28)	26 (22, 28)	–

No significant difference between rural and tribal women for any given nutrient as tested by regression analysis

[a] Estimated average requirement (EAR) for women 19–50 years old using USA values where available [31]

[b] Values adjusted for retention

between rural and tribal women for any of the nutrients presented in Table 3 (all $p>0.05$).

The primary food group that contributed to energy, protein, zinc, and phytate was cereals (Table 4). Over 64 % of energy in the women's diet came from cereals. Added fat, miscellaneous, and legumes food groups contributed around 12.7, 8.0, and 6.6 % of energy intake, respectively. The meat, fish, poultry, and egg food group contributed <1 % of energy in the women's diets. Similarly, for protein, just over 64 % came from cereals and 16 % came from legumes. Meat, fish, poultry, egg, and vegetable food groups contributed over 5 % of protein. Dairy foods contributed over 5 % of energy and protein, while fruits contributed <1 % of these nutrients. The majority of zinc came from the cereals food group (73 %) followed by legumes (8 %) and vegetables (7 %). The meat, fish, and poultry food groups contributed 2.5 % of total zinc. Phytate predominantly came from the cereals food group (86 %) followed by legumes (10 %).

Table 5 presents the non-dietary and dietary factors thought to be associated with serum zinc concentration as determined by unadjusted analysis. The non-dietary factors that were significantly related to serum zinc concentration were currently lactating and years of education at school. All of the dietary factors were significantly associated with serum zinc concentration apart from the phytate/zinc molar ratio and iron. In the non-dietary factor adjusted regression model, only currently lactating was significantly associated with serum zinc concentration. In the dietary factors regression model, none of the variables tested were significantly associated with serum zinc, although the phytate/zinc molar ratio and iron were approaching significance. The combined non-dietary and dietary factors final multiple regression model are outlined in the columns of the far right of Table 5. The two factors that were significantly associated with serum zinc were currently lactating (p=0.012) and energy intake (MJ) (p<0.001). It is estimated from this final model that breastfeeding (i.e., current lactation) decrease was associated with 7 % lower serum zinc concentration (95 % CI 2 to 12 %) compared to those women who were not breastfeeding. In addition, for every 1 MJ higher energy, serum zinc concentration was 2 % lower (95 % CI 1 to 3 %).

Discussion

This study of a representative sample of non-pregnant rural and tribal women in Ramtek Block, central India, clearly demonstrates that these women were at elevated risk of zinc

Table 4 Percent of energy, protein, zinc, and phytate intake from nine major food groups consumed by women from central India (n=103)

	Cereals	Dairy	MFP and egg	Legumes	Nuts, peanuts, and seeds	Fruit	Vegetables	Added fat	Miscellaneous
Energy	64.5	2.3	1.0	6.6	0.4	1.0	3.5	12.7	8.0
Protein	64.6	4.4	5.6	16.2	0.7	0.7	5.4	0.0	2.3
Zinc[a]	72.5	4.4	2.5	8.4	0.8	0.9	7.1	0.0	3.3
Phytate	85.7	–	–	10.4	1.3	0.2	1.1	0.0	1.2

MFP meat, fish, and poultry

[a] Values adjusted for retention

Table 5 Factors associated with serum zinc concentrations in women of reproductive age from central India

Variable	Ratio[a] (95 % CI) unadjusted model	p value	Ratio[a] (95 % CI) adjusted models[b]	p value	Ratio[a] (95 % CI) final model[c]	p value
Non-dietary[d]						
Age (5 years)	0.98 (0.93, 1.04)	0.412				
Total household income	1.00 (1.00, 1.00)	0.489				
Vegetarian	0.96 (0.89, 1.04)	0.299				
Married	0.98 (0.90, 1.06)	0.565				
Parity	0.98 (0.94, 1.03)	0.425				
Non-fasted	0.94 (0.86, 1.02)	0.133	0.95 (0.87, 1.04)	0.240		
Family members	0.99 (0.99, 1.00)	0.187	1.00 (0.97, 1.04)	0.781		
Education (years)	0.99 (0.98, 0.99)	0.036	0.99 (0.99, 1.00)	0.071	0.99 (0.98, 1.00)	0.122
Tribal community	0.94 (0.86, 1.03)	0.165	0.95 (0.88, 1.02)	0.129	0.94 (0.87, 1.01)	0.083
BMI	0.99 (0.97, 1.00)	0.089	0.99 (0.98, 1.00)	0.158	0.99 (0.98, 1.00)	0.104
Currently lactating	0.91 (0.84, 0.99)	0.033	0.90 (0.84, 0.96)	0.003	0.93 (0.88, 0.98)	0.012
Dietary[e]						
Energy (MJ)	0.97 (0.96, 0.99)	0.001	0.97 (0.94, 1.01)	0.116	0.98 (0.97, 0.99)	<0.001
[Phytate]:[zinc][f]	1.00 (0.99, 1.00)	0.060	0.99 (0.99, 1.00)	0.056	0.99 (0.99, 1.00)	0.232
Protein (10 g)	0.96 (0.94, 0.99)	0.004	0.99 (0.94, 1.04)	0.615		
Calcium (100 mg)	0.98 (0.97, 0.99)	0.044	1.00 (0.98, 1.02)	0.899		
Iron (mg)[f]	0.99 (0.98, 1.00)	0.059	1.00 (0.99, 1.02)	0.606		
Zinc (mg)[f]	0.98 (0.96, 0.99)	0.010				
Phytate (1000 mg)[f]	0.91 (0.86, 0.97)	0.009				

Participants with elevated CRP>6 mg/L (n=6) and use of oral contraceptives (n=1) have been removed from univariate and multivariate analysis

[a] Ratio of geometric means

[b] Variables included in the adjusted non-dietary model were non-fasted, family members, education, tribal community, BMI, and currently lactating. Variables included in the adjusted dietary model were energy, phytate/zinc ratio, protein, calcium, and iron. Only the phyate/zinc ratio was included in the adjusted and final model because this was considered a better predictor than either zinc or phytate

[c] Variables included in the final model were education, tribal community, BMI, currently lactating, energy, and phytate/zinc ratio

[d] n=102

[e] n=96

[f] Nutrients adjusted for retention

deficiency; using IZiNCG guidelines, a high percentage of women had an inadequate zinc intake (78 %) and a low serum zinc concentration (52 %). The median zinc intake of rural women was 5.6 mg/day, slightly lower than the mean zinc intake of 6.0 mg/day reported by Pathak et al. [11] in nulliparous rural women in Haryana, and higher than the median zinc intake of 4.7 mg/day in rural women reported by Agte et al. [12]. The median zinc intake of tribal women in the present study was 5.3 mg/day, which was higher than the median intake of tribal women of 3.1 mg/day reported by Agte and colleagues [12]. However, the sample size in the latter study was small (i.e., rural women (n=28) and tribal women (n=28)) and participants were surveyed in health camps; thus, these results are not likely to be representative of most rural and tribal women. The overall median zinc intake in the present study, was 5.3 mg/day, well below the IZiNCG EAR of 9 mg/day.

The proportion of women with inadequate zinc intakes in the present study was 78 % (i.e., using the IZiNCG cutoff) although this should be interpreted with caution. IZiNCG suggests that usual intakes be adjusted for within-subject day-to-day variability, which narrows the distribution of intakes [32]. This is typically done by collecting a repeat diet recall and adjusting for day-to-day within-subject variation [33]. Due to logistic constraints, it was impractical to measure more than a single day's dietary intake in the women in this study; therefore, intakes were not adjusted. The distribution of zinc intakes will be wider than if intakes were adjusted, resulting in an underestimation of the prevalence of women with intakes below the EAR. This said, our results were not dissimilar to those of Pathak et al. [11], who reported 75.7 % of nulliparous rural Indian women had zinc intakes <50 % of the recommended dietary allowance (RDA), although this does not give an estimate of the proportion of low zinc intake. However, a review by Hotz [32] estimated that 50 % of women had inadequate zinc intakes or intakes below the EAR in the study by Pathak et al. The present study estimated that 72 % of rural and 82 % of tribal women were at elevated risk of low zinc intakes. Regardless of the actual prevalence, the true prevalence in this population will be well above the 25 % the IZiNCG cutoff, thus of public health concern with intervention advised [1].

The serum zinc results also lend support to the view that women in Ramtek Block were at elevated risk of zinc deficiency. Just over half of the women (52 %) had low serum zinc, which lies within the range of other serum zinc findings in rural and tribal Indian women [11, 34]. According to IZiNCG, >20 % of the population with a low serum zinc is indicative of an elevated risk of zinc deficiency [1]. Additional evidence of the elevated risk of zinc deficiency is supported by the dietary data. Women in this study had very low energy intakes and consumed only 5.4 MJ/day. A lack of energy in the diets in these women is reflected in the magnitude of chronic energy deficiency (CED); of the 63 % of women who had some form of CED (BMI <18.5 kg/m^2), 47 % had severe CED (i.e., BMI <16 kg/m^2). Although physical activity level was not directly measured, given the amount of physical work undertaken by women living in this area, an estimated 8.2 MJ/day would be required to meet their energy requirements [35]. In addition to physical work, temperatures of 40 °C or more in the summer months could also increase energy requirements. All of the Indian national surveys [6–10] have reported higher energy intakes of rural and tribal women than the women in this study, but our results are consistent with a smaller study of rural and tribal women in Western India [12]. Median intakes of protein, calcium, and iron were all very low. A low protein intake will also have an effect on zinc intake, as foods that are high in protein are often high in zinc [36].

IZiNCG states that the primary cause of zinc deficiency is likely to be due to inadequate intake of absorbable zinc [1]. Women in our study consumed a high amount of phytate, a known potent inhibitor of zinc absorption [37]. IZiNCG estimates that ~25 % of zinc is absorbed from diets based on unrefined cereals with a phytate/zinc molar ratio >18 [1]. The phytate/zinc molar ratio in the present study was 26. Phytate intake was only measured in one other Indian study [12] which reported a median phytic acid consumption of 581 and 341 mg for rural and tribal women, respectively, and a phytate/zinc molar ratio of ~12, although no details were given on how phytate intakes were determined [12]. In our study, the median phytate intake of women was 1,261 and 1,278 mg/day in rural and tribal women, respectively. Phytate may be partially degraded during cooking, therefore, its inhibitory effect may be diminished [38]. Phytate intakes may have been overestimated in the present study as raw food items were imputed from the food composition tables. Although the true phytate/zinc molar ratio may be lower than 26, it is still likely to be >18 given that the diet of women in this study was based on unrefined cereals. Notwithstanding, the results of the present study fill an important gap in this area and identify the main sources of phytate in the diets of these central Indian women.

The primary source of phytate consumed by the women in our study was cereals and legumes, which was also the food group contributing the most zinc in the diet. This source of zinc will not be as readily absorbed by the body. In contrast, meat, fish, and poultry are the richest food sources of zinc but only contributed 2.5 % of total dietary zinc. Vegetables contributed a similar percentage of zinc to the diet as did legumes, which might be an important strategy to improve zinc intake if any future dietary diversification/modification studies were to be conducted because vegetables have a lower phytate content than cereals and legumes. Furthermore, the majority of protein intake came from cereals and legumes and only 5.6 % came from meat, fish, poultry, and egg. This is of concern because the inhibitory effect of phytate on zinc has shown to be lessened by the presence of animal protein [36]. The NNMB surveys and other studies that examined food groups consumed by rural and tribal Indian women support the current findings that consumption of meat is low and unrefined cereal consumption is high by the women in this area [6–10, 39, 40]. This dietary pattern is very different from that observed in other countries, such as the USA, which is characterized by lower amounts of cereals and legumes and higher amounts of meats, eggs, and dairy products. The food group that made the largest contributions to the energy intake in these Indian women was cereals (64.5 %), a value more than twice the ~30 % of total energy coming from cereals in the usual American diet [41]. The high cereal content of the diet of Indian women also contributes the highest amount of protein (also 64.6 %) while meat, fish, poultry, and eggs only contributed 5.6 % of protein intakes; in contrast, cereals contribute ~15 % and meat, fish, poultry, and eggs ~43 % to total protein intake in the USA [41].

The main proportion of food sources that contribute to zinc intake in the present study is also similar to those of rural women from other low-income countries [42, 43]. These women live in extremely poor households, so it is not surprising that zinc intakes and consequently serum zinc concentration are low. It is clear that with limited financial resources these women have little means to buy zinc- and iron-rich animal flesh foods. Although these women classified themselves to be “non-vegetarian,” in reality, most are practicing vegetarianism. This is reflected by the low overall percentage of energy that comes from the meat, fish, poultry, and egg food group, which was only 1 %.

In the adjusted linear regression analysis, only breastfeeding (i.e., current lactation) and energy intakes were significantly associated with serum zinc concentration. Women who were breastfeeding had a 7 % lower serum zinc concentration compared to those women who were not currently breastfeeding. This finding is consistent with the data from NHANES II [44] and highlights the need for higher zinc intakes in breastfeeding women. Interestingly, a negative association was found between serum zinc concentration and energy intake. For each megajoule increase in energy consumed by the women, serum zinc concentration was 2 % lower. As the majority of energy in these women’s diets came from cereals and legumes, increasing energy intakes may reflect a diet that contains proportionately higher quantities of cereals and legumes. These foods are low in bioavailable zinc and also contain very high amounts of phytate resulting in a high phytate/zinc molar ratio, which could further inhibit zinc absorption and utilization. This result highlights that these Indian women need to consume more food, particularly more foods rich in zinc such as meat and fish, and proportionally less phytate-containing foods such as unrefined cereals.

The study was associated with a number of limitations including the relatively small sample size, logistic constraints that prevented the collection of 24-h dietary recalls on Saturdays and in other seasons than summer. Errors may also have arisen when zinc and phytate values were imputed from other food composition databases and may not reflect zinc concentration in plant foods grown in Indian soils [45]; Chiplonkar and Agte [46] found an estimated 19.8 % difference between estimating dietary zinc intake using indirect analysis (i.e. Indian food composition database) and directly measuring zinc food in a laboratory. Despite these concerns, this study has a number of strengths. Dietary data was of a high standard and followed standard recommendations for dietary zinc intake analysis outlined by Gibson and Ferguson [15]. All research assistants were given extensive training, an opportunity to practice the techniques on each other to gain experience, and also specific supervised training in pilot testing of the questionnaires in the rural environment in an attempt to mitigate collection error. Another key strength of this study compared to other studies previously discussed was the proportion to population sampling method, which would give a representative sample of women in Ramtek Block. Blood collection and serum zinc analysis followed IZiNCG recommendations to avoid adventitious contamination.

The current findings add substantially to our understanding of the zinc status of rural and tribal Indian women. The agreement between the biochemical and dietary data demonstrates that these women are at high risk of zinc deficiency. It is also clear that these women were not consuming enough food, which is reflected in the high proportion of women in the study with CED and severe CED. In this region of India, rural women are just as vulnerable to malnutrition as tribal women in Ramtek Block and should receive government health support; this would improve the overall health, nutrition, and quality of life of all women in Ramtek Block. This study can be used as a platform to inform research of potential food vehicles for fortification or dietary diversification and modification strategies to improve overall energy, micronutrient and, in particular, zinc intake in undernourished women living in India and other developing countries.

Acknowledgments This study was supported by a University of Otago Research Grant and the Department of Human Nutrition's Performance Based Research Fund. We thank all the women and families who participated in this study; our dedicated research assistants Dr. Harish Wankhede, Jayasree Barai, Mamta Wankhede, Ritu Hadekar, Neelima Selukar, Vaishali Nagarare, Vaishali Patil, Sachin Paunikar, Raju Kalbhut, Vijay Panchdane, Rupali Nimbekar, and Atul Dandekar; Ramtek Block Primary Health Centre medical officers and staff; and Ramtek Block ICDS staff.

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