

# Zinc Deficiency Is Common among Healthy Women of Reproductive Age in Bhaktapur, Nepal<sup>1,2</sup>

Ram K. Chandyo,<sup>3,4</sup> Tor A. Strand,<sup>4,6\*</sup> Maria Mathisen,<sup>4</sup> Manjeswori Ulak,<sup>3</sup> Ramesh K. Adhikari,<sup>3</sup> Bjørn J. Bolann,<sup>5,7</sup> and Halvor Sommerfelt<sup>4,8</sup>

<sup>3</sup>Department of Child Health, Institute of Medicine, Tribhuvan University, 2533 Kathmandu, Nepal; <sup>4</sup>Centre for International Health, and <sup>5</sup>Institute of Medicine, University of Bergen, 5020 Bergen, Norway; <sup>6</sup>Medical Microbiology, Department of Laboratory Medicine, Sykehuset Innlandet, 2609 Lillehammer, Norway; <sup>7</sup>Laboratory of Clinical Biochemistry, Haukeland University Hospital, 5021 Bergen, Norway; and <sup>8</sup>Division of Infectious Disease Control, Norwegian Institute of Public Health, 0403 Oslo, Norway

## Abstract

Zinc deficiency is a major public health problem in many developing countries. However, its prevalence is still unknown in most populations. Women of reproductive age in developing countries are highly vulnerable to nutritional deficiencies, including that of zinc. To estimate the prevalence of zinc deficiency and to identify important dietary sources of zinc, we undertook a cross-sectional survey in 500 nonpregnant Nepalese women and measured their plasma zinc concentrations. We also examined the associations between plasma zinc and dietary intake of zinc or phytate, iron status, plasma concentrations of C-reactive protein, albumin, and hemoglobin. Food intake was estimated by 2 24-h dietary recalls and 1 FFQ for each woman. The plasma zinc concentration was (mean  $\pm$  SD)  $8.5 \pm 2.4 \mu\text{mol/L}$  and more than three-quarters of the women were zinc deficient. Dietary zinc intake did not predict plasma zinc concentration, whereas phytate intake was negatively and significantly associated with plasma zinc. The other variables that were associated with plasma zinc were plasma albumin and hemoglobin concentration. Rice contributed 50% to the total estimated daily zinc intake and wheat and meat each contributed 15%. Rice also contributed 68% to the daily intake of phytate. In conclusion, we found that zinc deficiency was common in women of reproductive age and that the foods contributing substantial amounts of zinc also contributed importantly to the intake of phytate. *J. Nutr.* 139: 594–597, 2009.

## Introduction

Zinc is an essential trace element with a key role in numerous basic cellular functions in humans. It is crucial to the normal function of the immune system (1,2) and is involved in DNA synthesis, cellular division, proliferation, and growth (3). Zinc is also required during pregnancy for optimal growth and development of the fetus and for maternal tissue expansion (4). Poor maternal zinc status has been associated with negative pregnancy outcomes (5–7), including spontaneous abortion, congenital malformation, low birth weight, and preterm delivery (8–10). Micronutrient deficiencies in early pregnancy, including that of zinc, are common among Nepali women (11).

Traditionally, the Nepali diet is monotonous and cereal based and consists of limited amounts of food from animal sources. Cereal-based diets are high in phytate, which inhibits zinc absorption and the inhibitory effect is particularly high

when the phytate:zinc (P:Z)<sup>9</sup> molar ratio in the diet is  $>15$  (12).

Data on zinc deficiency based on population surveys are still lacking from many developing countries (13,14). Less precise estimates, such as those based on national food balance sheets and on the prevalence of clinical manifestations of zinc deficiency, like stunting and diarrhea in children, have been used instead (15). However, these proxies are influenced by several factors and are rather unspecific markers of zinc deficiency and probably not suitable for studying an adult population.

Our objective in this study was to assess the prevalence of zinc deficiency by measuring plasma zinc concentrations in a random sample of 500 women of childbearing age living in Bhaktapur, Nepal. We also measured plasma albumin and C-reactive protein (CRP) concentrations, because most of the intravascular zinc is bound to albumin and the concentration of plasma zinc is influenced by inflammation (16). In a subsample, we also administered 2 24-h dietary recalls to identify important sources of zinc and phytate.

<sup>1</sup> Supported by Norwegian Universities Committee for Development, Research and Education grant number 36/2002 and the Research Council of Norway grant numbers 160854 and 172226.

<sup>2</sup> Author disclosures: R. Adhikari, B. Bolann, R. Chandyo, M. Mathisen, H. Sommerfelt, T. A. Strand, and M. Ulak, no conflicts of interest.

\* To whom correspondence should be addressed. E-mail: tor.strand@cih.uib.no.

<sup>9</sup> Abbreviations used: CRP, C-reactive protein; CF, carpet factory; GAM, generalized additive model; ICP-AES, inductively coupled plasma atomic emission spectrometry; P:Z, phytate:zinc.

## Subjects and Methods

The study was approved by the ethical board of Institute of Medicine, Tribhuvan University in Katmandu, Nepal and the Human Research Ethics Committee of the Medical Faculty at the University of Bergen, Norway.

**Study area and food habits.** From September 2000 to November 2001, we recruited women from the Bhaktapur municipality in the Kathmandu valley, Nepal. This is a semiurban, agricultural-based town with 80% of the population constituted by the Newar ethnic group. Around the town of Bhaktapur, there are ~50 carpet factories (CF) in which migrant families from different ethnic groups, mainly Tamang and Magar, live and work for longer or shorter periods. The CF workers have become an important part of the population in Bhaktapur and were therefore also included in our study.

**Sample size.** We expected a prevalence of zinc deficiency of >25%. A sample size of 450 women is required to detect this prevalence with a lower 95% confidence limit of 21%. We assumed that we would be unable to obtain an adequate blood specimens from ~10% of the women and therefore targeted a sample size of 500.

**Selection procedures and dietary recalls.** The details of the selection procedures and dietary recall methods are provided elsewhere (17). Because of frequent migration, we were concerned that CF women would be underrepresented. To ensure that this important and marginalized group was adequately represented, we used separate sampling frames for the CF women and for the local residents.

The study included nonpregnant women aged 13–35 y, without any ongoing disease, who were living in the Bhaktapur municipality. Pregnancy status was assessed by asking about the date of the last menstruation and by a urine test for pregnancy, whenever necessary. We excluded women with acute (e.g. fever, diarrhea, dysentery) or chronic (e.g. tuberculosis, diabetes, hypertension) illness and those taking vitamins, minerals, or drugs (with the exception of hormonal contraceptives). From the lists of women in the 2 strata, we randomly selected and approached 792 women. We enrolled 500 of these women, 403 of whom were from the stratum consisting of local residents and 97 from the CF stratum. In 379 of the 500 enrolled women, we also administered a FFQ and 2 24-h dietary recalls ~1 wk apart and on different weekdays. The daily intakes of the various nutrients from the 24-h dietary recalls were calculated using Indian food tables from the Wfood2 program version 1.0 (18). Ideally, all the study subjects should be fasting before blood was collected, but this was not possible. We recorded the time of the last meal or snack before the women visited the clinic. The women were weighed using a UNICEF electronic scale (SECA) with an accuracy of 100 g and the height was measured using a locally made wooden board that measured height to the nearest cm.

**Laboratory analysis.** Blood was collected from the cubital vein between 0900 and 1500 (72% of the specimens before noon) in micronutrient-free heparinized polypropylene tubes (Sarstedt). Within 10 min of collection, the heparinized blood was centrifuged (760 × g; 10 min, room temperature), separated, and the plasma transferred to micronutrient-free polypropylene vials (Eppendorf). These vials were initially refrigerated at the field clinic for a maximum of 5 h, transported on ice to the university hospital the same day, and stored at –45°C until they were transferred on dry ice to Norway.

After thawing, the plasma specimens were analyzed for zinc using inductively coupled plasma atomic emission spectrometry (ICP-AES) from Thermo Jarell-Ash at the Laboratory for Clinical Biochemistry, Haukeland Hospital, Bergen, Norway. Spectrascan Certified Element Standard for Atomic Spectroscopy (Teknolab) was used as the reference standard. All specimens were analyzed twice and the mean concentration was used. The CV between the analyses was <6.5%. Plasma CRP and albumin levels were measured using an immunoturbidimetric (Tina-Quant, Roche) and a Bromocresol Green colorimetric assay, respectively, on a Modular P analyzer (Roche Diagnostics). Two vials contained too little material to obtain reliable zinc concentrations.

**Definitions.** Zinc deficiency was defined as a plasma zinc concentration <11.3 μmol/L for samples obtained in the morning from fasting women,

<10.7 μmol/L for samples obtained in the morning from women who did not fast, and <9.3 μmol/L for samples obtained in the afternoon (15). The estimated average requirement of zinc using an unrefined plant based diet is 9 mg/d for women aged 13–18 y and 7 mg/d for nonpregnant women older than 18 y (19). Women consuming less than these cutoffs were considered to have inadequate intake. The P:Z molar ratio in the diet reflects the inhibitory effect of phytate on zinc absorption and was estimated using a standard algorithm (20,21).

**Statistical analysis.** The data were double entered into Microsoft VisualFoxPro databases with computerized logic, range, and consistency checks. The associations between plasma zinc and the variables of interest were described by the Spearman correlation coefficients.  $P < 0.05$  was considered significant.

Descriptive statistics and linear regression analyses were undertaken using Stata, version 9 (STATA Corp) and, when appropriate, adjusted for the design effects induced by stratification and clustering. The figure describing the relationship between plasma zinc and plasma albumin concentration was constructed using generalized additive models (GAM) in the statistical software R, version 1.9.0. We also undertook crude and multiple GAM analyses to assess whether any of the associations were linear or confounded by other variables (22). Values in the text are means ± SD unless otherwise noted.

## Results

The plasma zinc concentration did not differ between local resident and CF women and this stratification variable did not modify any of the associations described in this article. The pooled data are therefore presented.

**Subject characteristics.** A total of 296 (59%) women were married and, among these, 209 (71%) used contraceptives, mainly Depo-Provera. The weight of the participants was  $48.8 \pm 7.5$  kg and their height was  $149.6 \pm 5.8$  cm; 86 (17%) of the women were shorter than 145 cm. Thirty-five (7%) of the women were fasting (no meals, snack, or tea before sampling) and one-third reported that they did not have a morning meal prior to blood sample collection. Among the women who reported having a morning meal (67%), the duration between the last meal and blood collection was 2.3 h (range, 0.55–6.25 h). Similarly, 60% of women reported having had tea, whereas 22% had a snack before blood sampling (Table 1).

**TABLE 1** General characteristics of the nonpregnant women included in a study on zinc status in Bhaktapur, Nepal<sup>1</sup>

Characteristics	
Age, y	23 ± 6
Married, n (%)	296 (59)
Hemoglobin, g/L	132 ± 13
Hemoglobin <120 g/L, n (%)	58 (12)
Plasma ferritin <15 μg/L, n (%)	98 (20)
Plasma CRP, mg/L	0 (0, 0.8)
Land owner, n (%)	333 (67)
Schooling, y	4 (0, 8)
BMI, kg/m <sup>2</sup>	21.8 ± 3.0
Smoker, <sup>2</sup> n (%)	26 (7)
Illiterate, n (%)	166 (33)
Daily wage earners, n (%)	247 (49)
Vegetarians, <sup>2</sup> n (%)	10 (3)

<sup>1</sup> Values are means ± SD,  $n = 500$  unless otherwise indicated,  $n$  (%), or median (interquartile range).

<sup>2</sup> Information based on 379 women from whom we obtained dietary recalls.

**TABLE 2** Plasma concentrations of zinc and albumin and intakes of zinc and phytate in nonpregnant women in Bhaktapur, Nepal<sup>1</sup>

Variables	Values
Plasma zinc concentration, $\mu\text{mol/L}$	8.5 $\pm$ 2.4
<11.3 $\mu\text{mol/L}$ in morning fasting samples, %	88 (29 of 33)
<10.7 $\mu\text{mol/L}$ in morning nonfasting samples, %	90 (291 of 324)
<9.3 $\mu\text{mol/L}$ in afternoon samples, %	78 (110 of 141)
Plasma albumin, g/L	42.5 $\pm$ 3.1
<35 g/L, %	1 (0.2)
Intake of nutrients, $n = 379$	
Zinc, mg/d	8.6 $\pm$ 3.3
<7 mg/d, % (95% CI) (women >18 y)	29 (23, 34)
<9 mg/d, % (95% CI) (women 13–18 y)	69 (58, 79)
Phytate, mg/d	2198 $\pm$ 695
P:Z molar ratio	26.4 $\pm$ 5.9

<sup>1</sup> Values are means  $\pm$  SD,  $n = 500$  unless otherwise indicated.

**Nutrient intake and P:Z molar ratio.** Seventy-six percent (95% CI: 72%, 81%) of the women had energy intakes less than the recommended dietary allowance of 9205 kJ. The intake of zinc and phytate and the P:Z molar ratio are presented (Table 2). The interquartile range of zinc intake was 7.2–9.4 mg. A total of 29% of women >18 y of age and 69% of women  $\leq$ 18 y of age had an inadequate intake of zinc. We present the main sources of zinc and phytate and their intake frequency (Table 3). Rice contributed 50% to the total daily zinc intake and wheat and meat each contributed 15%. Rice also contributed 68% to the intake of phytate. The P:Z molar ratio in our study was 26.4  $\pm$  5.9. The P:Z molar ratio was >15 in >90% of the women.

**Plasma zinc and its relation with plasma albumin and intake of zinc and phytate.** The plasma zinc concentration was 8.5  $\pm$  2.4  $\mu\text{mol/L}$  and the 2.5th and 97.5th percentiles were 5.3 and 14.1  $\mu\text{mol/L}$ , respectively. Overall, depending on fasting status, 78–90% of the specimens had plasma zinc concentrations that indicated zinc deficiency (Table 2). We depict the association between plasma zinc and plasma albumin concentration (Fig. 1).

Zinc intake was not associated with plasma zinc concentration in our study. The only nutrient intake that was associated with plasma zinc concentration was phytate, which was negatively correlated ( $r = -0.15$ ;  $P = 0.003$ ). However, the P:Z molar ratio was not associated with the plasma zinc concentration.

**TABLE 3** Main sources of zinc and phytate in nonpregnant women in Bhaktapur, Nepal<sup>1</sup>

Foods	Zinc	Phytate	Recalls with particular foods <sup>2</sup>	Women who reported	Zinc contribution	Phytate contribution
				consuming food item at least once a week <sup>3</sup>		
	mg/100 g			$n$ (%)	%	
Rice grain, flake, or flour	1.1	353–786	758 (100)	394 (100)	50	68
Wheat grain or flour (refined and unrefined)	2.0–2.3	620–845	314 (41)	255 (65)	15	18
Meat (buffalo, chicken, goat)	3.2	0	142 (19)	159 (40)	15	0
Green or dry vegetables (mustard, radish, spinach, etc.)	0.1–0.8	20–42	517 (68)	319 (81)	6	5
Pulses and beans (lentil, black and red gram)	0.8–1.3	255–358	294 (39)	256 (65)	3	3
Potatoes	0.3	81	569 (75)	347 (88)	3	4
Milk products (buffalo or cow)	0.3	0	500 (65)	259 (66)	3	0
Eggs	1.2	0	69 (9)	130 (33)	1	0

<sup>1</sup> Values are means, ranges,  $n$ , or percentages.

<sup>2</sup> Total 758 dietary recalls (2 recalls from each woman).

<sup>3</sup> Data based on FFQ from 394 women.

**Plasma zinc status and its relation with iron status and intake of iron.** Intakes of iron and zinc were strongly correlated ( $r = 0.79$ ;  $P < 0.001$ ). The Spearman rank correlation coefficients between zinc intake and plasma ferritin, plasma transferrin receptor, and hemoglobin concentration were 0.17 ( $P = <0.001$ ),  $-0.10$  ( $P = 0.056$ ), and 0.26 ( $P < 0.001$ ), respectively. The plasma zinc concentration was associated with plasma hemoglobin ( $r = 0.16$ ;  $P < 0.001$ ) but not with plasma transferrin receptor ( $r = -0.01$ ;  $P = 0.9$ ) or ferritin concentrations ( $r = 0.07$ ;  $P = 0.08$ ).

**Multiple regression analyses.** The crude associations presented here were not substantially altered when the independent variables were included in multiple regression models. The results from the multiple regression models are therefore not presented.

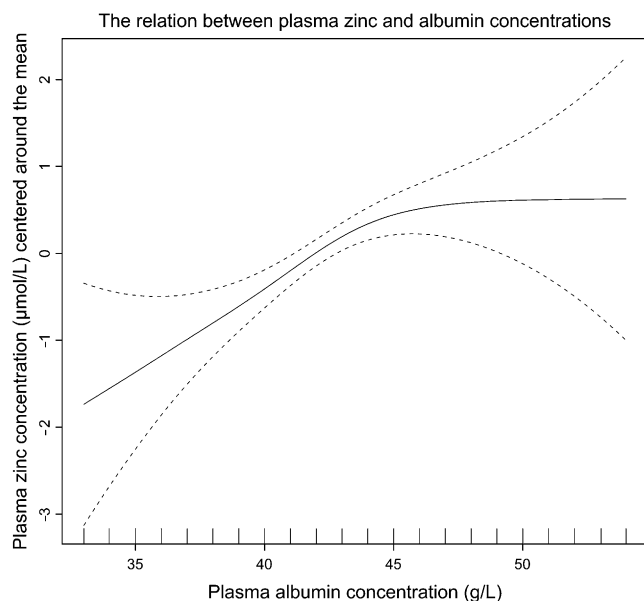
## Discussion

The results of our study indicate that more than three-quarters of the apparently healthy, nonpregnant women were zinc deficient as defined by low plasma zinc concentration. Similar prevalences of zinc deficiency have been reported among nonpregnant (23) and pregnant (11,24) Indian and Nepalese women.

The prevalence of iron deficiency anemia has been suggested as a proxy for zinc deficiency, because meat and other animal flesh foods are rich sources of both minerals (25). However, in this population, the prevalence of anemia and iron deficiency (17) was substantially lower than the prevalence of zinc deficiency. This was also found in a recent study in Ethiopian women (26).

We measured plasma zinc levels using ICP-AES. Although a study by Dipietro et al. (27) demonstrated a satisfactory agreement between ICP-AES and atomic absorption spectrometry, which is more frequently used for determining plasma zinc concentrations, we cannot rule out the possibility that ICP-AES and atomic absorption spectrometry give somewhat different readouts and, accordingly, different prevalence estimates.

The P:Z molar ratio was very high in our study, indicating a potential for profound inhibition of intestinal zinc absorption. Most of the intake of phytate was from rice (68%) and the phytate content was estimated from uncooked rice. Phytate is to some extent lost during cooking and we might accordingly have overestimated the phytate content in the food. However, a study from India suggests that cooking induces only a limited lowering of phytate concentration (28). The intake of zinc in our study



**FIGURE 1** Relation between plasma zinc and albumin concentration in nonpregnant women in Bhaktapur, Nepal,  $n = 500$ . The graph was generated using a GAM in R. The solid lines depict the regression line from the GAM analysis and the areas between the broken lines represent the 95% CI of these regression lines.

was not associated with plasma zinc concentration. Plasma zinc reflects an individual's usual zinc intake over a few weeks or months (29). Meat, which in this population was the food item that had the highest concentration of bioavailable zinc, was consumed by most women but not on a regular basis. This relatively high intra-person variability in consumption of zinc-dense foods in combination with the very high level of phytate intake could result in a weaker association between zinc intake or the P:Z molar ratio with plasma zinc. Furthermore, we have used Indian food tables from Wfood2 to calculate the intake of zinc (18). The zinc and phytate content of the local foods are probably somewhat different, but we think that this is the best available tool in the absence of Nepalese food composition tables.

In conclusion, our study indicates that there is a high prevalence of zinc deficiency in women of reproductive age in Bhaktapur, Nepal. This may increase the risk of infections and poor pregnancy outcomes in these women. Moreover, food that contributed most to the intake of zinc also contributed substantially to the intake of phytate, which seemed to have a negative impact on their zinc status.

### Acknowledgments

We thank Shyam S. Dhaubhadel, founder chairman of Siddhi Memorial Hospital in Bhaktapur, for his cooperation in undertaking the study and Irene Ro Iversen at the Laboratory for Clinical Biochemistry at Haukeland University Hospital for proficient processing of the plasma specimens.

### Literature Cited

- Fraker PJ, King LE, Laakko T, Vollmer TL. The dynamic link between the integrity of the immune system and zinc status. *J Nutr.* 2000;130:S1399–406.
- Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr.* 1998;68:S447–63.
- MacDonald RS. The role of zinc in growth and cell proliferation. *J Nutr.* 2000;130:S1500–8.

- King JC. Determinants of maternal zinc status during pregnancy. *Am J Clin Nutr.* 2000;71:S1334–43.
- Leek JC, Vogler JB, Gershwin ME, Golub MS, Hurley LS, Hendrickx AG. Studies of marginal zinc deprivation in rhesus monkeys. V. Fetal and infant skeletal effects. *Am J Clin Nutr.* 1984;40:1203–12.
- Keen CL, Lonnerdal B, Golub MS, Uriu-Hare JY, Olin KL, Hendrickx AG, Gershwin ME. Influence of marginal maternal zinc deficiency on pregnancy outcome and infant zinc status in rhesus monkeys. *Pediatr Res.* 1989;26:470–7.
- Osendarp SJ, West CE, Black RE. The need for maternal zinc supplementation in developing countries: an unresolved issue. *J Nutr.* 2003;133:S817–27.
- Prasad AS. Zinc deficiency in women, infants and children. *J Am Coll Nutr.* 1996;15:113–20.
- Caulfield LE, Zavaleta N, Shankar AH, Meriardi M. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. *Am J Clin Nutr.* 1998;68:S499–508.
- Seshadri S. Prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in South East Asia. *Br J Nutr.* 2001;85 Suppl 2:S87–92.
- Jiang T, Christian P, Khattry SK, Wu L, West KP Jr. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. *J Nutr.* 2005;135:1106–12.
- Lonnerdal B. Dietary factors influencing zinc absorption. *J Nutr.* 2000;130:S1378–83.
- Chakravarty I, Sinha RK. Prevalence of micronutrient deficiency based on results obtained from the national pilot program on control of micronutrient malnutrition. *Nutr Rev.* 2002;60:S53–8.
- Black RE, Sazawal S. Zinc and childhood infectious disease morbidity and mortality. *Br J Nutr.* 2001;85: Suppl 2:S125–9.
- International Zinc Nutrition Consultative Group (IZiNCG). Assessing population zinc status with serum zinc concentration. Technical Brief. No 2. 2007.
- Strand TA, Adhikari RK, Chandyo RK, Sharma PR, Sommerfelt H. Predictors of plasma zinc concentrations in children with acute diarrhea. *Am J Clin Nutr.* 2004;79:451–6.
- Chandyo RK, Strand TA, Ulvik RJ, Adhikari RK, Ulak M, Dixit H, Sommerfelt H. Prevalence of iron deficiency and anemia among healthy women of reproductive age in Bhaktapur, Nepal. *Eur J Clin Nutr.* 2007;61:262–9.
- Wfood2. World food 2 computer software package. Version 1.0. Berkeley (CA): The Regents of the University of California. 1996.
- International Zinc Nutrition Consultative Group (IZiNCG). Determining the risk of zinc deficiency: assessment of dietary zinc intake. Technical brief No. 3. 2007.
- Gibson RS, Ferguson E. An Interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries. Washington (DC): International Life Sciences Institute; 1999.
- WHO. Trace elements in human nutrition and health. Geneva: WHO; 1996.
- Wood S. Modelling and smoothing parameter estimation with multiple quadratic penalties. *J R Statist Soc B.* 2000;62:413–28.
- Pathak P, Kapil U, Kapoor SK, Dwivedi SN, Singh R. Magnitude of zinc deficiency among nulliparous nonpregnant women in a rural community of Haryana State, India. *Food Nutr Bull.* 2003;24:368–71.
- Kapil U, Pathak P, Singh P, Singh C. Zinc and magnesium nutriture amongst pregnant mothers of urban slum communities in Delhi: a pilot study. *Indian Pediatr.* 2002;39:365–8.
- Hotz CB, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. *Food. Nutr Bull.* 2004;25:S130–61.
- Gibson RS, Abebe Y, Stabler S, Allen RH, Westcott JE, Stoecker BJ, Krebs NF, Hambidge KM. Zinc, gravida, infection, and iron, but not vitamin B-12 or folate status, predict hemoglobin during pregnancy in Southern Ethiopia. *J Nutr.* 2008;138:581–6.
- Dipietro ES, Bashor MM, Stroud PE. Comparison of an inductively coupled plasma-atomic emission spectrometry method for the determination of calcium, magnesium, sodium, potassium, copper and zinc with atomic absorption spectroscopy and flame photometry methods. *Sci Total Environ.* 1988;74:249–62.
- Agte VV, Tarwadi KV, Chiplonkar SA. Phytate degradation during traditional cooking: significance of the phytic acid profile in cereal based vegetarian meals. *J Food Compost Anal.* 1999;12:161–7.
- Hess SY, Peerson J, King J, Brown K. Use of serum zinc concentration as an indicator of population zinc status. *Food Nutr Bull.* 2007;28:S403–29.