Original Research

Predicting Bioavailable Zinc from Lower Phytate Forms, Folic Acid and Their Interactions with Zinc in Vegetarian Meals

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Key words: zinc bioavailability, prediction model, vegetarian meals, folic acid, phytate form

Objective: To develop a statistical model for predicting zinc bioavailability from cereal-based vegetarian meals using relative proportion of nutrients, non-nutrients and their interactive effects.

Methods: A database on in vitro zinc dialysability (by isotopic tracer, ^{65}Zn) of vegetarian meals (266 out of 326) from Asia, Africa, Europe /US and Latin America was used to develop a model for estimating zinc bioavailability. A multiple regression analysis adjusted for energy content was carried out for net bioavailable zinc from a meal with the predictor variables as meal contents of iron, zinc, copper, ascorbic acid, β-carotene, riboflavin, thiamine, folic acid, tannic acid, fiber, phytate degradation products (IP6 to IP1), along with their interaction terms. Reproducibility of the model was tested with remaining 60 meals. Validation of the model was done with zinc absorption data of i) 12 young adults on 24 meals and ii) 5 adults with ileostomy on 7 meals.

Results: Folic acid, IP3 and IP5 were significant influencing factors for bioavailable zinc. Weighted multiple regression equation was: ln (bioavailable zinc in mg) $= -1.701 + 1.285 \times$ ln [(IP5 in mg) $\times$(Zn in mg)] $-1.222 \times$ ln(IP5 in mg) $-0.0078 \times$ folic acid in μg $-0.137 \times$ ln [(IP3 in mg) $\times$(Zn in mg)] with adjusted R^[2] = 0.64, p = 0.0001. The correlation between predicted and observed dialysability of meals was found to be 0.96 ($p < 0.01$). A significant correlation between observed and predicted amount of absorbed zinc (r = 0.85, $p < 0.01$) was obtained for the human data of zinc absorption in 12 healthy and 5 subjects with ileostomy.

Conclusions: Bioavailable amount of zinc from vegetarian meals was influenced by IP3, IP5 and folic acid content and their interactive effect with zinc content.

INTRODUCTION

In recent years, zinc has received a greater attention in human health due to its involvement in many body processes and its antioxidant properties. Though cereals, corn and vegetables contain large amounts of zinc, populations subsisting on cereal-based diets are predicted to be at high risk of zinc deficiency. This is because same foods also contain large quantities of the non-nutrient inositol phyates, which are known to be potent inhibitors of zinc absorption from composite meals [1]. With elimination of meat and increased intake of phytate-containing legumes and whole grains, the absorption of both iron and zinc is lower with vegetarian than with non-vegetarian diets [2]. It is therefore postulated that vegetarians may have as much as a 50% higher need for zinc than non-vegetarians [3]. Experimental studies have identified a number of dietary factors as potential promoters or antagonists of zinc absorption [4]. To examine adequacy of dietary zinc in vegetarians it is worthwhile to consider the bioavailable zinc intake rather than gross intakes.

Bioavailability of zinc is enhanced by dietary protein, but plant sources of protein are also generally high in phytic acid [1,5–7]. The effect of phytate is, however, modified by the source and amount of dietary proteins consumed. [8]. Various other dietary factors such as iron, calcium, folic acid, riboflavin and niacin have been identified to affect bioavailability of zinc [1,3,5,9,10]. Also interactive effects between iron and zinc, calcium and zinc have been observed. A competitive interaction between iron in supplements and zinc may limit zinc absorption, although no effect is observed when iron and zinc are consumed in a meal [11,12]. Similarly when zinc intake is

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adequate, intake of a calcium-rich diet has little effect on zinc absorption [3]. Thus relative proportion of the dietary factors, both nutrients and non-nutrients, are likely to play a major role in determining zinc bioavailability. Moreover, these factors may interact with each other and resultant amount of bioavailable zinc is not the mere sum of bioavailable amounts from individual ingredients in the diet.

Studies reporting algorithms for predicting zinc bioavailability are limited and have not incorporated interaction terms. It has been postulated that the [Ca] × ([phytate]/[Zn]) ratio can be used as a predictor of zinc bioavailability [13]. Although there are studies that support this concept, the interaction is complex, and it is possible that this ratio may be of limited predictive value [1]. Phytate-to-zinc molar ratio has been advocated to estimate zinc bioavailability from the diet [14], and it is possible that this ratio in general may have more predictability than the ratio that includes calcium as a variable. A nonlinear regression, assuming the exponential form between fractional absorption of zinc and phytate intake or phytate:zinc molar ratio of maize tortillas with different phyatate content has been postulated [15]. However relationship of dietary factors in a composite vegetarian meal having possible enhancers and inhibitors of zinc availability needs to be investigated.

Many studies have reported that reducing phytate (IP6) to lower ester forms (IP5 to IP1) through methods such as leavening of bread, fermentation, germination, milling or addition of phytase to the diet enhanced zinc absorption [16–20]. Inositol compounds with three to five phosphate groups (IP3-IP5) showed binding affinities for zinc at neutral pH with relative concentrations that had been found in a range of students' meals [21]. Therefore "total phytate" of a meal is an inadequate measure when evaluating zinc bioavailability [22]. Instead, individual phytate forms (IP6-IP1) need to be considered for prediction of zinc absorption.

The assessment of zinc deficiency is a complicated task [23,24]. A simple tool to assess sub optimal zinc status may be offered by predicting bioavailable zinc intakes through a statistical model, which includes dietary factors and their interactions influencing zinc absorption. This paper, therefore, presents a modeling approach for predicting zinc bioavailability from cereal-based vegetarian meals. For this, a large database on zinc dialysability of 266 composite vegetarian meals out of 326 meals was used [25]. An algorithm was developed to predict net bioavailable zinc from a meal with the predictor variables as amounts of other dietary factors and their interaction effects. The developed algorithm was validated by the remaining data on 60 meals.

Our earlier study has established a good agreement between in vitro and in vivo estimation of bioavailability of iron and zinc [26]. In vivo validation of the new algorithm was done by two human studies of zinc absorption in a) 12 healthy adults and b) 5 adults with ileostomy.

MATERIALS AND METHODS

Selection of Meals/Diets

Vegetarian meals were formulated using cereals, legumes, fruit and vegetables based on observed proportions of daily consumption patterns in India (National Nutrition Monitoring Bureau, India (NNMB, 1991) [27] and diet patterns reported in literature representing food habits of people in different regions of the world from Food encyclopedia [28]. In all, 248 Indian, 25 other Asian, 24 Europe/USA, 12 Latin American, and 17 African vegetarian meals were selected. Meals comprised of any one or a combination of six cereals (wheat, rice, sorghum, pearl millet, finger millet and maize) along with any one or more of the foods viz.; legumes, leafy vegetables, other vegetables, fruits, milk products, oil, spices and sugar (Table 1). Details are given elsewhere [25]. Out of these 326 meals, 60 meals representing all the regions were selected by stratified random sampling technique and set aside for validation of the model. Estimation of percent dialysability was obtained with ^{65}Zn, by standard protocol simulating gastro intestinal conditions [29].

Preparation of Meals

Foods were prepared in a traditional manner as observed in each region. Indian meals were prepared with items like unleavened whole flour pancakes (roti), pressure-cooked rice, legume curries using either tamarind juice, jaggery, coconut or curry powders (spices), vegetables either by shallow pan-frying using onion, chilli, salt and oil or as curries. The details are reported earlier [19]. Other continental meals were prepared using canned foods, bakery products from super market for international customers and by mixing the food items in observed/reported proportions.

Estimation of Nutrient Contents and Zinc Dialysability of the Meals

Cooked meals were homogenised and analysed in triplicates for nutrient contents in the laboratory using various techniques described in NIN manual [30]. β-carotene, folic acid, and vitamin C were estimated by spectrophotometry, Thiamine and riboflavin were estimated flourometrically as described earlier [31]. Contents of zinc, iron, copper of the meals were estimated by dry ashing and atomic absorption spectophotometry (UNICHEME, India). Protein contents were estimated using micro Kjeldahl method and fat contents by Soxlet extraction. Ash was estimated by igniting food sample in the muffle furnace at 550° C. Energy value of meals was estimated using proximate principles and the Atwater's factors. Tannins were estimated using Folin-Denis reagent [32]. Fibre was estimated by detergent system [33] and hemicellulose was estimated by difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF). Levels of phytic acid degradation products (IP1, IP2, IP3, IP4, IP5 and IP6) were estimated by the method reported previously [19].

Mean content of energy, protein, fat, 8 micronutrients, tannins, fiber and phytates of 326 meals has been given in Table 2a and 2b. Mean energy content of different cereal-based meals ranged from 101 to 132 kcal/100g cooked weight. Mean zinc contents varied from 0.41 to 0.62 mg per 100 g cooked weight of the meal and total phytate-P content ranged from 11.8 to 23.7 mg.

Cooked meals were homogenised to a desired consistency of 10 g dry matter/100 ml. Six aliquots each of 20 gm were taken in a plastic container and each aliqot was spiked with radioactive ^{65}Zn (37 KBq or 1 μci in 25% HCl). Segments of dialysis tubing containing 25 ml water and amount of 0.5 M $NaHCO_3$ equivalent to titratable acidity measured previously were placed in each container. After digestion under simulated gastrointestinal conditions, radioactivity in the dialysates and undialysable part in the container was determined by counting 10 ml samples in gamma counter (Electronic Corporation of India) in duplicates. Percent dialysability was the ratio of dialyzable to total aliquot. Details are given elsewhere [26,29].

Dialysable zinc density (D_{Zn}) was computed as:

D_{Zn} = (mg of bioavailable zinc/total energy content) × 1000

Dialysable zinc for different meals has been given in Table 2a and 2b. Energy content of total meals ranged from 290 to 1810 kcal.

Initially a simple additive model was developed to estimate total bioavailable zinc from a meal. In vitro dialysability data from 20 individual foods and 40 cereal-based meals using these 20 foods was used for this purpose [29]. Dialysability of these meals was computed by weighted average of individual food's dialysability. The computed zinc dialysability was significantly smaller (5.39 ± 0.90%) than the experimental value (9.7 ± 3.5%) as revealed by paired t-test ($p<0.01$). This indicated that net zinc bioavailability is not the simple addition of the bioavailable zinc of the ingredients and there is a need for a systematic model building with interaction terms.

Data For in Vivo Validation of the Model

For validation of the model *in vivo,* two previous studies of zinc absorption (34, 35) were used.

1) Study 1: Metabolic experiment was carried out for zinc balance on 12 young healthy adult males (19.2 ± 0.7 yr) who volunteered for the study. Mean weight of these subjects was 52.7 ±1.2 kg and height 164.2±5.3 cm. A habitual daily diet of these subjects contained items such as unleavened pancake of wheat flour (chapatti, around 250 to 300 g), rice (60–80 g), split redgram or greengram (40 g), any one legume such as moth bean, pea, cowpea or lentil (50 g), vegetables like brinjal, ladies' finger, cluster beans (40 g), onion (40 g), tomato (100 g), carrot/beat/cucumber (45 g), one fruit banana/orange/chiku, milk (50–75g), sugar/jaggery (15 g), and oil (40 g) for cooking. Their mean daily energy intake was 2533±191 kcal/day. Composite samples of diets and pooled samples of feces for one week were dry ashed and extracted with AR grade concentrated HCl and glass-distilled water and analysed for zinc content by atomic absorption spectrophotometry (Perkin-Elmer USA, model 2308). Average percent absorption of zinc was 15.8 ± 4.2. The method used and the menus given were described in detail previously [34].

2) Study 2: Subjects were adults (45.6±8.1 yr) who underwent ileostomy operation but were not taking radiotherapy or any other medicines. Meals chosen were from their habitual dietary

Table 1. Composition of Meals for Estimation of in Vitro Zinc Dialysability

Name of Cereal (No of meals)	Amounts of ingredients of the meals							
	Cereal (g)	Legumes (g)	GLV (g)	Other vegetables (g)	Fruit (g)	Milk & milk products (g)	Oil (g)	Sugar/ Jaggery (g)
Wheat (36)	113 ± 23	7.3 ± 4.3	7.1 ± 3.2	23.9 ± 6.8	12.3 ± 7.0	33.2 ± 12.1	4.1 ± 1.1	3.1 ± 1.2
Sorghum (20)	222 ± 27	6.4 ± 2.6	13.4 ± 5.6	26.2 ± 11.1	5.8 ± 5.0	28.1 ± 18.8	9.0 ± 2.1	2.6 ± 1.5
Pearl millet (21)	217 ± 14	11.6 ± 5.2	14.8 ± 5.9	23.6 ± 7.6	13.4 ± 6.7	31.1 ± 15.9	6.8 ± 1.7	6.7 ± 2.2
Rice (71)	204 ± 13	28.4 ± 3.6	7.8 ± 2.0	41.6 ± 5.9	31.7 ± 8.6	35.2 ± 11.4	9.1 ± 1.6	7.5 ± 1.3
Finger millet (17)	210 ± 10	8.1 ± 4.9	19.1 ± 7.0	18.2 ± 7.6	13.7 ± 9.2	10.6 ± 8.5	4.6 ± 1.1	1.8 ± 1.0
Maize (24)	143 ± 18	17.3 ± 5.1	13.8 ± 5.4	13.5 ± 6.0	10.4 ± 6.3	10.3 ± 8.3	3.1 ± 0.5	2.4 ± 1.4
Rice-wheat (85)	67 ± 7 158 ± 9	60.0 ± 3.5	9.0 ± 3.3	100.7 ± 8.4	54.4 ± 5.2	33.5 ± 5.1	20.9 ± 1.2	15.7±2.3
Rice-sorghum (29)	96 ± 4 136 ± 19	31.2 ± 4.2	16.4 ± 8.0	43.9 ± 9.2	13.0 ± 8.1	76.8 ± 26.8	14.0 ± 1.6	15.4±2.9
Rice-pearl millet (10)	120 ± 24 237 ± 61	40.7 ± 10.9	7.5 ± 5.3	53.3 ± 13.2	26.9 ± 14.0	86.1 ± 59.6	16.1 ± 5.3	22.1 ± 5.8
Rice-finger millet (4)	117 ± 18 212 ± 48	40.0 ± 9.7	3.8 ± 2.4	24.7 ± 9.2	6.5 ± 3.8	–	5.1 ± 1.7	12.1 ± 4.2
Miscellane ous (9)	129 ± 16	7.8 ± 7.7	–	3.1 ± 2.9	9.8 ± 8.8	26.1 ± 20.1	1.0 ± 0.9	4.7 ± 4.1

Values represent mean ± SE.

Table 2a. Average Nutrient Contents of Single Cereal Meals per 100 G Cooked Weight and Average Percent Zinc Dialysability, Bioavailable Zinc Density Dzn (Mg/1000 Kcal)

Nutrient	Wheat-based meals	Sorgum-based meals	Pearl millet-based meals	Rice-based meals	Ragi-based meals	Maize-based meals
Energy (kcal)	119 ± 8	118 ± 8	110 ± 7	127 ± 6	101 ± 7	103 ± 6
Protein (g)	3.2 ± 0.22	3.3 ± 0.33	3.0 ± 0.31	2.9 ± 0.22	2.3 ± 0.4	2.7 ± 0.4
Fat (g)	1.7 ± 0.15	2.1 ± 0.25	1.9 ± 0.41	1.8 ± 0.14	1.3 ± 0.1	1.9 ± 0.7
Iron (mg)	1.0 ± 0.1	1.1 ± 0.12	1.4 ± 0.11	0.92 ± 0.07	0.85 ± 0.06	0.70 ± 0.06
Zinc (mg)	0.41 ± 0.03	0.49 ± 0.05	0.62 ± 0.06	0.57 ± 0.05	0.46 ± 0.04	0.47 ± 0.04
Copper (mg)	0.15 ± 0.01	0.16 ± 0.02	0.20 ± 0.02	0.16 ± 0.01	0.12 ± 0.02	0.14 ± 0.02
β-Carotene (μg)	396 ± 89	700 ± 202	635 ± 194	285 ± 68	925 ± 246	872 ± 209
Ascorbic Acid (mg)	3.3 ± 0.5	5.5 ± 1.1	4.5 ± 0.9	4.4 ± 0.5	6.0 ± 1.5	4.8 ± 1.0
Riboflavin (μg)	30 ± 4	32 ± 3	29 ± 3	31 ± 2	36 ± 8	26 ± 5
Thiamine (μg)	76 ± 8	66 ± 9	79 ± 13	86 ± 6	57 ± 8	70 ± 10
Folic acid (μg)	13.3 ± 1.7	14.6 ± 3.0	21.6 ± 2.8	11.9 ± 1.3	18.3 ± 3.3	15.2 ± 2.9
Tannic acid (mg)	1.9 ± 0.2	3.7 ± 0.4	1.9 ± 0.4	1.2 ± 0.1	4.7 ± 0.7	3.6 ± 0.4
Total fibre (g)	2.7 ± 0.26	3.1 ± 0.2	4.0 ± 0.4	3.2 ± 0.3	4.0 ± 0.3	3.1 ± 0.3
Hemei-cellulose (g)	1.2 ± 0.15	1.5 ± 0.1	1.5 ± 0.2	0.91 ± 0.07	2.3 ± 0.4	2.0 ± 0.2
Total phytate-P (mg)	12.6 ± 1.5	16.8 ± 1.6	16.5 ± 1.2	11.8 ± 1.0	14.6 ± 1.4	11.9 ± 0.1
IP1 (mg)	0.29 ± 0.05	0.41 ± 0.10	0.33 ± 0.06	0.44 ± 0.06	0.26 ± 0.05	0.23 ± 0.04
IP2 (mg)	0.63 ± 0.2	1.1 ± 0.3	0.78 ± 0.14	0.91 ± 0.13	0.58 ± 0.08	0.43 ± 0.05
IP3 (mg)	1.4 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
IP4 (mg)	1.6 ± 0.2	2.1 ± 0.3	2.1 ± 0.3	1.8 ± 0.2	1.7 ± 0.2	1.5 ± 0.2
IP5 (mg)	2.5 ± 0.3	3.4 ± 0.5	3.5 ± 0.4	2.5 ± 0.3	2.7 ± 0.3	2.2 ± 0.3
IP6 (mg)	6.2 ± 0.8	7.9 ± 0.4	8.1 ± 0.6	4.7 ± 0.3	8.1 ± 0.8	6.3 ± 0.5
% dialysability	16.7 ± 1.2	20.2 ± 3.1	15.3 ± 1.4	17.1 ± 0.8	19.8 ± 2.3	20.4 ± 2.2
DZn	0.61 ± 0.06	0.85 ± 0.15	0.85 ± 0.10	0.74 ± 0.05	0.92 ± 0.15	0.95 ± 0.14

Values represent mean ± SE.

pattern. Meals were ad libitum and composition is given in Table 3. Contents of nutrients, phytate forms (IP1 to IP6), fiber (NDF, ADF), tannins of the meals and ileostomy fluid were estimated in the laboratory as described above. Zinc absorption was measured as difference of zinc content of the meal and the ileostomy fluid and average percent zinc absorption was 18.8±5.4. Details of the methods are reported elsewhere [35].

Table 2b. Average Nutrient Contents of Mixed Cereal Meals Per 100 G Cooked Weight and Average Percent Zinc Dialysability, Bioavailable Zinc Density (Mg/1000 Kcal)

Nutrient	Wheat-Rice-based	Sorghum-Rice-based	Pearl-millet-Rice	Finger-millet-Rice
Energy (kcal)	120 ± 2	113 ± 6	132 ± 12	116 ± 14
Protein (g)	3.7 ± 0.12	3.7 ± 0.3	4.0 ± 0.4	3.1 ± 0.5
Fat (g)	2.7 ± 0.13	1.6 ± 0.2	2.0 ± 0.4	1.8 ± 0.3
Iron (mg)	1.15 ± 0.03	1.0 ± 0.07	1.2 ± 0.14	1.14 ± 0.18
Zinc (mg)	0.52 ± 0.02	0.61 ± 0.05	0.51 ± 0.07	0.59 ± 0.13
Copper (mg)	0.24 ± 0.01	0.14 ± 0.01	0.2 ± 0.04	0.16 ± 0.04
β-Carotene (μg)	87 ± 4	102 ± 10	113 ± 16	113 ± 16
Ascorbic Acid (mg)	4.3 ± 0.4	3.8 ± 0.5	4.6 ± 1.1	3.7 ± 0.8
Riboflavin (μg)	26 ± 1	29 ± 2	27 ± 4	22 ± 4
Thiamine (μg)	47 ± 3	71 ± 6	65 ± 11	72 ± 24
Folic acid (μg)	12.2 ± 0.8	8.9 ± 1.6	8.8 ± 2.5	9.7 ± 2.3
Tannic acid (mg)	1.1 ± 0.04	0.9 ± 0.08	1.5 ± 0.3	2.0 ± 0.3
Total fibre (g)	3.0 ± 0.1	3.3 ± 0.2	4.1 ± 0.4	3.0 ± 0.4
Hemei-cellulose (g)	1.2 ± 0.1	1.4 ± 0.15	1.1 ± 0.2	0.4 ± 0.1
Total phytate-P (mg)	23.7 ± 1.2	13.7 ± 0.9	12.1 ± 1.6	12.0 ± 2.6
IP1 (mg)	0.92 ± 0.08	0.44 ± 0.05	0.40 ± 0.06	0.38 ± 0.08
IP2 (mg)	1.81 ± 0.14	0.79 ± 0.08	0.81 ± 0.15	0.84 ± 0.23
IP3 (mg)	3.25 ± 0.22	1.42 ± 0.12	1.35 ± 0.31	1.36 ± 0.27
IP4 (mg)	4.53 ± 0.25	2.24 ± 0.23	2.14 ± 0.30	1.84 ± 0.47
IP5 (mg)	5.28 ± 0.38	2.6 ± 0.24	2.3 ± 0.27	2.88 ± 0.78
IP6 (mg)	7.9 ± 0.27	6.2 ± 0.41	5.1 ± 0.68	4.7 ± 1.02
% dialysability	15.1 ± 0.6	15.5 ± 0.8	18.2 ± 2.0	14.6 ± 1.3
DZn	0.65 ± 0.03	0.85 ± 0.09	0.75 ± 0.15	0.77 ± 0.19

Values represent mean ± SE.

Table 3. Composition and Contents of Energy, Micronutrients, Phytate Forms and Folic Acid Content of Meals of Ileostomy Subjects

Meal No	Ingredients	Energy (kcal)	Protein (g)	Zinc (mg)	IP3 (mg)	IP5 (mg)	Total phytate-P (mg)	Folic acid (μg)
1	Rice (125), Wheat roti (110), Brinjal (31), Fenugreek leaves (22), Cauliflower (20), Field beans (40)	566	12.6	2.0	9.1	11.3	80.8	34.0
2	Rice (62), Split green gram (138), Green peas (60), Wheat roti (106), orange (50)	629	16.2	2.5	9.2	11.0	195.7	38.5
3	Wheat roti (130), Mix legumes (88), Rice (92), Fenugreek leaves (52), Papaya (200), Green peas (26)	782	21.1	3.1	13.1	14.2	118.7	48.4
4	Wheat roti (28), Pulav (116), Fenugreek leaves (23), Papaya (160)	243	6.5	0.8	3.3	3.7	29.9	18.3
5	Wheat roti (164), Ladies'finger (76), Rice with split greengram (104)	522	12.9	2.2	5.6	7.1	76.6	32.9
6	Sorghum roti (152), fenugreek leaves (284), moth beans (284)	316	5.6	1.2	3.1	4.9	32.0	25.2
7	Wheat roti (146), French beans (192), Drumstick (243), Tomato+cucumber+onion (51)	226	6.1	0.9	2.8	3.7	28.5	18.5

Statistical Methods

All the statistical analyses were carried out using SPSS 11.0 under Windows. Contents of known inhibitors and enhancers in each meal formed the set of explanatory variables while dialyzable zinc was the response variable in the multiple regression model. Total 17 variables (5 vitamin contents, 3 mineral contents, 6 phytate components, 2 fiber components and tannins) were considered for developing the model. Variables were tested for normality and homogeneity of variances. Non-normal variables were transformed before further statistical analysis. Initially relationships of these variables with the response variable, viz., dialyzable zinc, were studied through individual scatter plots and regressions for groups of related variables. The variables showing significant relationship with dialyzable zinc entered the final equation. Only those interaction terms were considered for the model, which were plausible from the knowledge of relationships reported in nutrition literature [1, 2, 3, 36]. To account for the differences in energy value of different meals, stepwise weighted least square multiple regression analysis was carried out for log transformed dialyzable zinc with the selected variables and all binary interaction terms after verifying non-collinearity. The developed model was validated; first with the *in vitro* availability of zinc in a separate data set of 60 meals and then with two previous studies on *in vivo* zinc absorption using paired t test and correlation coefficient.

RESULTS

Multiple Regression Model

Multiple regression analysis weighted for energy content of meals revealed that amongst the group of IP1, IP2, IP3, IP4, IP5, IP6, NDF, hemicellulose and tannic acid with due transformations of these variables, lnIP5 and lnIP3 were significant factors along with their interaction terms with zinc content. Similarly separate weighted multiple regression analysis was carried out for the group of vitamins and minerals. Folic acid, riboflavin, iron, zinc and calcium were retained as significant variables with riboflavin × zinc, folic acid × zinc and calcium × zinc interaction terms. A final weighted multiple regression model was fitted to the data with the significant variables and interaction terms from these two analyses. Following prediction equation emerged which could explain 64% of the variation in zinc bioavailability.

Ln (bioavailable zinc in mg) $= -1.701 + 1.285 \times \ln[(\text{IP5 in mg}) \times (\text{Zn in mg})] - 1.222 \times \ln(\text{IP5 in mg}) - 0.0078 \times$ folic acid in μg $- 0.137 \times \ln[(\text{IP3 in mg}) \times (\text{Zn in mg})]$ R^[2]=0.648, adjusted R^[2]=0.64, p=0.0001.

Validation of the Model Using in Vitro Data

Using the above equation, dialyzable amount of zinc was computed for the remaining 60 meals. The difference between observed and predicted dialyzable zinc was not statistically significant ($p>0.1$), and the correlation between them was 0.96 (Fig. 1).

Validation of Model Using Human Absorption Data

Applying the same equation, bioavailable zinc for 12 healthy adults and 5 adults with ileostomy was computed and compared with the observed zinc absorption (Fig. 2). There was a significant correlation between observed and predicted amount of absorbed zinc ($r=0.85$, $p<0.01$).

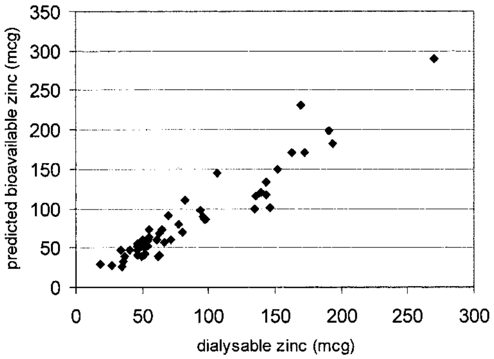

Fig. 1. Dialysable zinc vs amount of bioavialble zinc from algorithm. The figure represents validation of the model by in vitro dialysability of 60 meals. The correlation between the observed and predicted dialysability was r-0.96, $p<0.01$.

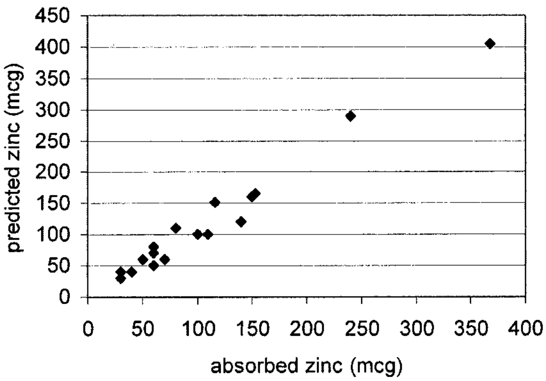

Fig. 2. Amount of zinc absorbed vs predicted zinc from algorithm. Figure of observed zinc absorption in 17 adults versus prediction by the proposed model exhibits a statistically significant correlation $r=0.85$, $p<0.01$.

DISCUSSION

The multiple regression model developed for predicting dialyzable zinc from a composite vegetarian meal contained terms of IP3, IP5, folic acid and the interaction between IP forms and zinc. There was a statistically significant correlation between observed dialysability of zinc from meals and that predicted from the model. Though the model was constructed using in vitro data, predicted values agreed well with human absorption data.

Our model can serve as a good index for assessing zinc adequacy in vegetarian and lacto-vegetarian diets. However it requires the estimates of IP3 and IP5 in the meals, which are generally not known for all the foods. Majority of food tables do not provide the estimates of even total phytate contents. Without these estimates the application of the model is not possible. We have estimated inositol phosphates (IP6-IP1) for over 300 individual foods, which contain preparations of cereal, legumes, vegetables, desserts and milk products. Using these data one can estimate the values of IP forms as also folic acid. In the present study the model was validated with a small number of young healthy adults. Such validation needs to be done with more number of individuals having diverse food habits.

Most of the available information on the effect of specific dietary factors on zinc absorption has been derived from single test meal studies [1]. However, zinc absorption from composite meal may have different influencing factors. Our earlier results have shown that the meal containing an enhancing factor should have least number of food ingredients in order to exert it's significant enhancing effect [25]. In vitro availability of zinc from a mixed meal was also been reported to be most sensitive to increased spiking of food constituents (coffee, vitamin C, wheat bran and pectin) than iron and calcium [37]. Present model reveals a dire need to include interactive effects of nutrients and non-nutrients for predition of zinc availability for a composite meal.

Total zinc content of a meal also influences the absorption of zinc. Specifically, percent absorption decreases with increasing intake of zinc, although the absolute amount of zinc absorbed increases. Therefore we felt it more appropriate to consider amount of bioavailable zinc as the dependent variable in the model rather than the percent zinc bioavailability. Multiple regression analysis adjusted for energy content has resulted in giving zinc content as the non-significant factor. The diets being cereal-legume based, the source of zinc and energy were the same. This probably explains absence of zinc content as one of the explanatory variable in our final model.

Minerals such as iron, calcium, copper & magnesium compete with zinc for availability [36, 38]. However none of these factors remained in the final model. This may be because our meals/diets were habitual containing indigenous sources of minerals in natural form. Moreover the contents of minerals were also not high as in supplements to hamper the zinc bioavailability. Supplemental (38–65 mg/day of elemental iron) but not dietary levels of iron are found to decrease zinc absorption [11,12]. Also more typical intakes of zinc do not affect copper absorption and high copper intakes do not affect zinc absorption [8]. Thus our results are in agreement with the fact that these minerals do not affect zinc absorption when taken in a meal rather than a high dose of supplements [3,39]. Therefore none of these minerals remained in the form of a significant factor or interaction term in the final model.

Protein was also not retained as the significant factor in our final equation. Studies on the effect of various protein sources are often confounded by the fact that the proteins contain other constituents that may affect zinc absorption. Animal protein (e.g., beef, eggs, cheese) has been shown to counteract the inhibitory effect of phytate on zinc absorption from single meals [7]. Milk protein, specifically casein in milk, has been shown to have a negative effect on zinc absorption [1]. In the present study, vegetarian meals contained meager amounts of milk and main source of protein was plant foods. Further adjusting for energy value of the meals might have resulted in protein to be non-significant in the final model.

Total "phytate" analysis, which includes inositol phosphates with varying degrees of phosphorylation, can give misleading information with regard to mineral availability. In addition, even limited dephosphorylation of inositol hexaphosphate can have a positive effect on mineral absorption [40]. Our model therefore used all the inositol phytate forms as probable influencing factors, taking into account the fact that lower forms are less inhibitory than higher phytate forms. This has been revealed in the model by the terms of IP3, IP5 and their interaction with zinc. However this does not indicate that IP4 and IP6 are not inhibiting the zinc absorption. But amongst IP1 to IP6 only IP3 and IP5 were found to be statistically significant and remained in the final equation. While selecting from IP1 to IP6 care was taken of the correlations between them and with bioavailable amount of zinc. A separate regression indicated IP3 and IP5 to be significant hence these terms were included in the final equation.

Amongst vitamins, only folic acid remained in the final equation. In our cell culture experiments, it was observed that in presence of folic acid the albumin bound zinc increases as compared to control, in turn reducing zinc uptake by cells [41]. Reduced zinc absorption has been reported to be one of the potent safety issues of folic acid supplementation [3,42]. Inclusion of folic acid as an inhibitor in the present model agrees well with these studies.

Estimates of dietary zinc absorption as developed by WHO have been stated as 50%, 30% and 15% for diets having phytate:zinc molar ratio less than 5, 5–15 and $>$15 respectively [43]. Average phytate:zinc molar ratio in our study meals was 11.1 ± 6.1 indicating estimate of zinc absorption to be 30%. However the observed value of average zinc absorption was 16.8% implying the need for a more sophisticated model.

In conclusion, our model highlights the importance of interrelationships between micronutrients such as vitamins, trace metals and non-nutrients i.e. phytate. It brought out the interaction of IP3, IP5 and folic acid with zinc bioavailability. It is hoped that this model will provide a better criterion for assessing adequacy of zinc in the vegetarian diets. Further research may be required for suitability of the model for prediction of human absorption in other settings and developing a model for non-vegetarian meals and compare it with the proposed model for vegetarian meals.

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