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The Current High Prevalence of Dietary Zinc Inadequacy among Children and Women in Rural Bangladesh Could Be Substantially Ameliorated by Zinc Biofortification of Rice[1–3]

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Abstract

Rural Bangladeshi populations have a high risk of zinc deficiency due to their consumption of a predominantly rice-based diet with few animal-source foods. Breeding rice for higher zinc content would offer a sustainable approach to increase the population's zinc intakes. The objectives of the study were to quantify usual rice and zinc intakes in young children and their adult female primary caregivers and to simulate the potential impact of zinc-biofortified rice on their zinc intakes. We measured dietary intake in a representative sample of 480 children (ages 24–48 mo) and their female caregivers residing in 2 rural districts of northern Bangladesh. Dietary intakes were estimated by 12-h weighed records and 12-h recall in homes on 2 nonconsecutive days. Serum zinc concentrations were determined in a subsample of children. The median (25th, 75th percentile) rice intakes of children and female caregivers were 134 (99, 172) and 420 (365, 476) g raw weight/d, respectively. The median zinc intakes were 2.5 (2.1, 2.9) and 5.4 (4.8, 6.1) mg/d in children and women, respectively. Twenty-four percent of children had low serum zinc concentrations ($<$9.9 μmol/L) after adjusting for elevated acute phase proteins. Rice was the main source of zinc intake, providing 49 and 69% of dietary zinc to children and women, respectively. The prevalence of inadequate zinc intakes was high in both the children (22%) and women (73–100%). Simulated increases in rice zinc content to levels currently achievable through selective breeding decreased the estimated prevalence of inadequacy to 9% in children and 20–85% in women, depending on the assumptions used to estimate absorption. Rural Bangladeshi children and women have inadequate intakes of zinc. Zinc biofortification of rice has the potential to markedly improve the zinc adequacy of their diets. J. Nutr. 140: 1683–1690, 2010.

Introduction

Zinc is essential for normal growth and immune function (1). In children residing in countries with an elevated risk of zinc deficiency, zinc supplementation enhances growth (2,3), decreases morbidity from diarrhea and pneumonia (3,4), and decreases mortality (3,5). Although zinc supplementation has been proven effective, coverage of zinc supplementation programs is low (5), and other sustainable methods of increasing zinc intakes should be explored. Plant breeding of staple crops, such as rice, to increase the zinc concentration is a promising approach to increase zinc intakes of populations in developing countries. Increases of ~8 μg zinc/g rice through conventional breeding are anticipated (6).

Rice is the staple of the diet in Bangladesh, contributing ~80% of dietary energy (7). Rice-based diets of the rural poor in Bangladesh are often low in animal-source foods and likely to be low in absorbable zinc. Nationally representative data on dietary zinc intakes or serum zinc concentrations are not available; however, an estimated 50% of the population is at risk of inadequate zinc intake based on national food supply data (1). The prevalence of child stunting, a functional indicator of population zinc status (8), is high in Bangladesh. According to 2007 national survey data, 43% of children $<$5 y are stunted

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[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

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[height-for-age Z-score (HAZ)[9] < -2 according to WHO standards] and the prevalence of stunting was highest among children 24–47 mo (53–54%) and among children residing in rural areas (9). The zinc status of Bangladeshi women is unknown; however, 33% of women residing in rural areas are underweight (9), indicative of low energy intakes and presumably proportionally low zinc intakes. High prevalences of low serum zinc concentrations in Bangladeshi children and women have been reported in some community-based research trials (10,11).

Among the initial steps to assess the potential impact of zinc-biofortified rice on zinc status of the population is to quantify rice and zinc intakes in representative samples of the target population. This paper will present results from a dietary intake survey in 2 rural rice-producing regions in Bangladesh. The primary objectives were to quantify rice and zinc intakes of young children and women and to simulate the potential impact of zinc-biofortified rice on zinc intake adequacy. In addition, we assessed the anthropometric status and serum zinc concentrations of the children to further appraise their risk of zinc deficiency.

Methods

Study design and population selection. The study was a cross-sectional, multi-stage, sample survey of dietary intakes of children 24–48 mo of age and their primary female caregivers. Two rural agricultural subdistricts in northern Bangladesh, Trishal Upazila in Mymengsingh District and Pirgacha Upazila in Rangpur District, were selected based on their high prevalence of poverty, food insecurity, and stunting, and a high share of production of the most common rice varieties. Trishal Upazila had a population of 372,500 and ~80,000 households, and Pirgacha Upazila had a population of 295,000 and ~72,000 households (12). The study population was selected using a multi-stage cluster sampling design. Within each of the 2 subdistricts, 25 clusters were selected using population-proportionate sampling based on estimates from 2001 census data; 24 clusters in each subdistrict were included in the study, with an extra cluster selected in case logistical problems prevented recruitment in 1 cluster. At the second stage of sampling, 10 households were selected within each cluster using a global positioning system sampling method. Two randomly selected starting points were selected in each cluster and transect lines were randomly determined from each starting point. The first 5 households in unique *baris* (extended families living in close vicinity) with a child 24–48 mo were identified by walking along each transect line. If a household refused to participate, another household was identified until the desired 5 households along each transect line agreed to participate. A total of 240 households from 24 clusters in each of the 2 subdistricts were selected. Ninety-two percent of the identified eligible households were enrolled in the study. Informed consent was obtained by study physicians. The study was approved by the ethical review committees at the University of California, Davis and the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B). The sample size allowed us to estimate usual rice intakes for children or women in each subdistrict with a precision of 12% and to estimate a 20% prevalence of low serum zinc concentrations with a precision of 7% with 95% confidence.

Field data collection procedures. Data collection took place from October 2007 to April 2008 in Trishal and from January to June 2008 in Pirgacha. Field workers visited the households in each cluster within a 1- to 2-wk period to assess dietary intakes, perform anthropometry, and administer demographic questionnaires. The field workers verified the ages of the children with immunization records.

Blood samples were obtained from a subset of children ($n = 280$) by selecting from the first 6 households who agreed in each cluster. Children were brought to a centralized location, such as a health center or school, within each cluster early in the morning. To standardize the time between a meal and blood sampling, children who were breast-feeding were allowed to breast-feed upon arrival and nonbreast-feeding children were given ~120 mL of cow milk. A venous blood sample was obtained 60 min after the milk feeding. The blood samples were collected in trace element-free vacutainers and kept cold until separation and processing at a field laboratory (1). The blood tubes were centrifuged at $1380 \times g$ for 5 min and the serum was stored in vials at −18 to −20°C.

The children were weighed nude or with light clothing on an infant balance (±5 g; Seca model 727). The women were weighed on an electronic scale (±0.1 kg; Seca 840 Digital Floor Scale). Height was measured to 0.1 cm using a ShorrBoard measuring board (Shorr Productions). Duplicate measures were obtained and averaged. If duplicate height measures differed by >0.5 cm, a 3rd measure was obtained and the 2 measures within 0.5 cm were averaged.

Dietary intakes of the children and their female caregivers were assessed by direct observation in the homes by trained local research assistants using 12-h weighed food records and recall of any foods consumed during the subsequent 12-h period. Two nonconsecutive days of dietary information were obtained during 1 wk, with 1 d on a weekend for most households. During the 12-h observation period, all food items, recipe ingredients, prepared recipes, and beverages served to the child and any uneaten portions were weighed using electronic balances (±1 g; MyWeigh KD7000). Meat, fish, and egg portions were weighed separately when served in mixed dishes rather than assuming the proportion was the same as in the prepared recipe to improve accuracy of intake of important sources of zinc. Amounts of foods consumed during the subsequent 12 h were assessed by recall using 2-dimensional plates and local cups and utensils to estimate portion sizes. For mixed dishes consumed during the recall period, if the recipe information was not observed during the 12-h observation, then the caregiver was asked to recall the amounts of ingredients and the total recipe weight was imputed from average recipe information from other households. If the caregiver could not recall amounts of ingredients in the recipe, then an average recipe was used.

Breast milk intake was estimated by the test weighing procedure. Children were weighed using an electronic infant balance (±5 g; Seca Baby Scale Model 727) before and after each feeding during 12 h of observation. The 12-h intake of breast milk was calculated by summing the differences in body weights of each feeding episode. The mean amount of breast milk per feed was calculated and multiplied by the number of feeding episodes reported during the subsequent 12-h recall period to estimate breast milk intake during the recall period. Insensible losses were measured by weighing the child before and after ~30 min when the child was resting. Corrections for insensible losses were made using the mean value of all children ($0.0465\ \text{g}\cdot\text{kg body weight}^{-1}\cdot\text{min}^{-1}$).

Foods consumed were converted to nutrients using food composition data from the USDA database (13), the WorldFood Dietary Assessment System (14), laboratory analyses, and the literature (15,16). Nutrient values for breast milk were obtained primarily from the literature (17–20). Energy content of breast milk was calculated using protein and carbohydrate values from a previous study in Bangladesh (17) and a calculated fat value based on laboratory-analyzed fatty acid concentrations of breast milk from women in our study (21). Food sources of energy and zinc were obtained by summing the total nutrient intakes for all child-days, summing the nutrient intakes from each food source, and calculating the percentage of total nutrient intake from each food.

Biochemical analyses. Serum samples (150 μL) were diluted (1:12) with distilled deionized water, and the zinc concentration was measured using atomic absorption spectophotometry (Shimadzu AA6501S). A standard curve was prepared from commercially available standard zinc nitrate solution (BDH). Bi-level control (UTAK Laboratories) and pooled serum were used to determine accuracy and precision. For

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[9] Abbreviations used: AGP, α1-acid glycoprotein; CRP, C-reactive protein; EAR, Estimated Average Requirement; HAZ, height-for-age Z-score; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh.

normal and high levels of zinc quality controls, the within-day CV were 1.9 and 1.3%, respectively, and the between-day CV were 9.0 and 5.7%, respectively. The within-day and between-day CV for zinc in the serum pool were 1.6 and 1.8%, respectively. Serum α1-acid glycoprotein (AGP) and serum C-reactive protein (CRP) were measured by immunoturbidimetric assay using commercial kits (Roche Diagnostics) and Hitachi 902 auto analyzer (Boehringer Mannheim Lab Diagnostics). Precinorm and Precipath (Roche Diagnostics) proteins and pooled serum were used to determine accuracy and precision of the AGP and CRP assays. For low and high levels of the AGP quality controls and for the serum pool, the within-day CV were 1.0, 0.4, and 0.8%, respectively, and the between-day CV were 1.9, 1.2, and 0.7%, respectively. For low and high levels of CRP quality controls and the serum pool, the within-day CV were 1.7, 1.1, and 5.6%, respectively, and the between-day CV were 1.8, 1.3, and 6.4%, respectively. All analyses were performed at the ICDDR,B.

Zinc and phytate composition of rice and lentil. We collected samples of rice and lentils from 1 household in each of the 24 clusters in each of the 2 sites for analysis of zinc and phytate content. Samples of 4–5 g of raw rice and 8–10 g of cooked rice from the same meal and 4–5 g of raw lentil were collected using stainless steel utensils and stored in acid-washed, trace element-free, plastic tubes. The moisture content of food samples was determined by calculating the difference in weight before and after drying at ~100°C for ~12–14 h at ICDDR,B. Zinc content of dried samples was determined by inductively coupled plasma atomic emission spectrometry (ARL 3580B, ARL) following nitric/perchloric acid digestion (22) at Waite Analytical Services, University of Adelaide, Australia. The analyzed mean zinc concentration of a certified reference material (NIST Corn Bran RM-8433, National Institute of Standards and Technology, Gaithersburg, MD) was 19.2 ± 0.2 μg/g (n = 6; CV = 1.2%) and within the certified range (18.6 ± 2.2 μg/g). Phytate content was determined by Dionex liquid chromatography (Dionex) using 200 mmol/L NaOH/deionized water as an eluant following extraction in 1.25% H_2SO_4 (23) at the School of Biological Sciences, Flinders University, Adelaide, Australia. The zinc and phytate contents of dried samples were adjusted for the moisture content of raw samples to yield values on a raw weight basis.

Data analysis. Data were entered into Microsoft Office Access files and verified by visual checks with the original data forms and by database queries. All statistical analyses were conducted using SAS software (version 9.2). Anthropometric Z-scores were calculated using the 2006 WHO growth standards and SAS macros provided by WHO (24,25). BMI of the women were calculated (kg/m^2) and classified according to WHO guidelines (26).

Individual serum zinc concentrations were adjusted for elevated acute phase proteins (indicators of systemic infection or inflammation). First, we conducted linear regression using a procedure to account for the clustered survey design (PROC SURVEYREG), with serum zinc concentrations as the dependent variable and independent variables that included the time of day of blood sampling and an indicator variable for elevated acute phase proteins (1,10). We defined elevated acute phase proteins as AGP ≥ 1.2 g/L (27) and CRP ≥ 5 mg/L (28). An indicator variable for infection status was used with 4 categories: *1*) normal AGP and CRP (reference category); *2*) elevated AGP and normal CRP; *3*) normal AGP and elevated CRP; and *4*) elevated CRP and AGP. Then, for children who fell into categories 2–4, the β-coefficient from that category was added to the child's analyzed serum zinc concentration to yield an adjusted value representative of a child with no infection (category 1). The prevalence of low serum zinc concentrations using a cutoff of 9.9 μmol/L (1) was determined from the adjusted concentrations, and the difference in prevalence between districts was assessed using PROC SURVEYLOGISTIC. Serum zinc concentrations were expressed as mean ± SD. Differences between districts in mean serum zinc concentrations were assessed using PROC SURVEYMEANS.

Distributions of usual rice and zinc intake were estimated by the National Cancer Institute method (29), which removes within-person variability of nutrient intake (30). Intake distributions were estimated by district and season and also for children by age group (24–35 or 36–48 mo) and breast-feeding status. Three seasons were defined in accordance with rice harvests: *1*) pre-Aman harvest (Oct–Nov); *2*) post-Aman harvest (Dec–Mar); and *3*) during Aus and Boro harvests (Apr–Jun). Effects of these factors on rice and zinc intakes were assessed using linear regression models (PROC SURVEYREG). We estimated absorbable zinc intakes of the women by using a prediction equation based on total zinc and phytate intakes (31). Zinc intakes were expressed as mean, median, and 25th–75th percentile.

We conducted simulations to examine the effect of increasing the zinc content of rice on zinc intakes. Separate random samples of 15, 35, and 70% of children and women were selected to simulate different levels of adoption of zinc-biofortified rice in the population. An additional 8 μg/g raw weight of zinc was added to the baseline zinc concentration of rice, an amount anticipated through conventional breeding (6), for those randomly selected individuals only. No change in the phytate content of zinc-biofortified rice was assumed. For each population adoption level, the National Cancer Institute method was used to estimate zinc intake distributions and prevalence of zinc intake inadequacy by the Estimated Average Requirement (EAR) cut-point method (30). For children, we defined total dietary zinc inadequacy using the EAR for 1- to 3-y-old children (<2 mg/d) (1). For women, we defined total dietary zinc inadequacy using 2 estimates of bioavailability: *1*) higher bioavailability with a mixed/refined vegetarian diet (<6 mg/d); and *2*) lower bioavailability with an unrefined, cereal-based diet (<7 mg/d); we defined absorbed zinc inadequacy as <1.86 mg/d (1).

Results

The total sample size was 479 households (data from 1 household were lost). Seventeen children had missing anthropometric data due to the child refusing to cooperate, no one at home on the measurement day, or lost data forms; the final sample size for anthropometric variables was 463 children. One child had an implausibly low value for HAZ based on WHO criteria (<−6 SD) (24) and was dropped only from the analyses using HAZ. Anthropometric measures were available for 468 women. Serum zinc concentrations were available for 279 of the children (one sample was hemolyzed). For dietary intake analyses, data from 16 children were dropped due to missing (n = 14) or implausible (n = 2) breast milk intake data, resulting in a total sample size of 463. Dietary data were available for 478 female caregivers (one caregiver was a male).

Characteristics of the children and their primary caregivers and households are described in **Table 1**. The mean age of the children was 35.5 mo and 50% of the children were still breast-feeding. Overall, 56% of the children were stunted (HAZ < −2), with a higher prevalence in Trishal, and 12% of children were wasted (weight-for-height Z-score < −2). The primary female caregivers of the children had low levels of formal education and high rates of illiteracy. Four percent of the female caregivers were grandmothers of the children.

Mean serum zinc concentrations of the children were 1.6 μmol/L lower in Trishal than in Pirgacha ($P < 0.0001$; **Table 2**). Correcting for elevated acute phase proteins increased the mean serum zinc concentration by 0.1 μmol/L, but the difference between districts remained the same. Overall, 24% of children had low serum zinc concentrations after correcting for elevated acute phase proteins; however, the prevalence was higher in Trishal (36%) than in Pirgacha (11%; P = 0.005). Thirty-four percent of children in Trishal had elevated acute phase proteins compared with 15% of children in Pirgacha. Children selected to provide a blood sample were 1.6 mo older than children who did not provide a sample (P = 0.02) but did not differ in anthropometric or sociodemographic characteristics.

District-specific zinc concentrations of rice samples collected from the homes were used in the analysis of dietary intakes, and a composite concentration from both districts was used for lentil

TABLE 1 Characteristics of children, their adult female primary caregivers, and their households in 2 rural districts of Bangladesh[1]

	Trishal	Pirgacha
n	239	240
Child characteristics		
Age,[2] *mo*	35.5 ± 7.0	35.5 ± 7.1
24.0 – 35.9, *%*	48.5	50.0
36.0 – 47.9, *%*	51.5	50.0
Male, *%*	56.7	56.3
Breast-feeding, *%*	50.2	50.8
Body weight, *kg*	11.2 ± 1.7	11.3 ± 1.5
Height, *cm*	86.5 ± 6.2	87.9 ± 5.6
Weight-for-age Z-score	−1.94 ± 1.00	−1.83 ± 0.96
Underweight (< −2), *%*	44.5	42.8
HAZ	−2.31 ± 1.15	−1.93 ± 1.20
Stunted (< −2), *%*	64.5	48.3
Weight-for-height Z-score	−0.89 ± 0.92	−1.07 ± 0.88
Wasted (< −2), *%*	11.0	12.3
Primary female caregiver characteristics		
Age, *y*	29.7 ± 8.8	26.1 ± 6.7
BMI,[3] *kg/m²*	19.4 ± 2.5	19.9 ± 2.7
Underweight (<18.5), *%*	38.5	35.4
Normal (18.5–24.9), *%*	59.3	58.2
Overweight (≥25.0), *%*	2.2	6.3
Education level, *%*		
No formal education	40.3	37.1
Class 1–4	21.0	10.6
Class 5	17.7	14.4
Class 6–9	14.7	24.5
>Class 9	6.3	13.1
Literacy, *%*		
Read	47.9	52.3
Write	39.5	49.4
Housing characteristics		
Type of walls, *%*		
Mat, cardboard, or plastic	2.5	0
Hemp, hay, or bamboo	13.0	40.9
Mud	28.2	1.3
Wood	1.3	1.7
Tin	49.2	46.4
Brick or concrete	5.5	8.9
Other	0.4	0.4
Electricity, *%*	30.3	27.0
Possessions, *%*		
Mobile phone	27.2	28.3
Radio	18.0	19.4
Television	25.1	27.0
Ceiling fan	22.2	21.9

[1] Data are mean ± SD or percent.

[2] Ten of the children were outside of the age range of 24.0–47.9 mo; 2 children in the first age category were 23.8–23.9 mo and 8 children in the second age category were 48.0–49.2 mo.

[3] BMI categories were classified according to WHO (26).

and for phytate concentrations of both rice and lentil. The median zinc concentrations of uncooked rice sampled from the homes were 0.92 and 0.76 mg/100 g raw weight in Trishal and Pirgacha, respectively. In cooked rice samples, they were 0.99 and 0.81 mg/100 g raw weight equivalent in Trishal and Pirgacha, respectively. The median zinc concentration of uncooked lentil was 3.55 mg/100 g raw weight. The median phytate concentrations were 121 mg/100 g raw weight for cooked rice and 212 mg/100 g raw weight for uncooked lentil.

The mean rice intake of the children and women were 138 g/d and 422 g/d raw weight, respectively (**Table 3**). The conversion factor for raw to cooked weight of rice is 2.99 based on an average of all observed rice recipes. Women in Pirgacha consumed 42 g raw weight/d more rice than the women in Trishal ($P = 0.002$). In a multivariate model of rice intake, children who were younger or breast-feeding had lower rice intakes (**Supplemental Table 1**). There was a slight trend in decreasing rice intakes among the children from the pre-Aman harvest to the post-Aman harvest and to the Aus/Boro harvests ($P = 0.07$). Seasonal rice intakes of the women were lowest in the pre-Aman harvest season.

Energy intakes of both children and women were higher in Pirgacha than Trishal (Table 3). In children, energy intakes were highest in October–November and there was a significant decreasing trend over the season ($P = 0.003$) intakes (Supplemental Table 1). In contrast, energy intakes in the women were lowest during October–November. The children's zinc intakes tended ($P = 0.005$) to be higher in Pirgacha than Trishal (Table 3). Children in Pirgacha consumed more zinc from animal sources than children in Trishal ($P < 0.0001$). Zinc intakes of the women did not differ by district. The women's mean fractional absorption of zinc was 25%. Women in Trishal had a slightly, but significantly, higher intake of absorbable zinc ($P < 0.0001$). Rice contributed 58% of energy and 49% of zinc to children's diets and 84% of energy and 69% of zinc to the women's diets (**Supplemental Table 2**).

Overall, 22% of the children had inadequate total zinc intakes and the prevalence was higher (25%) in Trishal than in Pirgacha (18%). The simulated effects of biofortified rice on dietary zinc intake adequacy are presented in **Table 4**. The prevalence of children's inadequate zinc intakes decreased by 3, 6, and 13 percentage points as the estimated population adoption levels increased to 15, 30, and 70%, respectively.

Overall, 73% of the women had inadequate total zinc intakes with the higher bioavailability assumption and the prevalence of inadequacy decreased by 18, 32, and 53 percentage points with the increasing estimated adoption levels. At a lower bioavailability diet assumption, 94% of the women had inadequate total zinc intakes and the prevalence of inadequacy decreased by 16, 32, and 57 percentage points with the increasing estimated adoption levels. All of the women had inadequate intakes of estimated absorbable zinc, and the estimated effect of zinc-biofortification on absorbable zinc intakes at the highest adoption level reduced the prevalence of inadequacy to 85%. The mean total zinc intakes of the women at the 3 adoption levels (15, 35, and 70%) were 6.0, 6.6, and 7.8 mg/d, respectively; the mean absorbable zinc levels at the 3 adoption levels were 1.34, 1.43, and 1.61 mg/d, respectively.

Discussion

In representative populations of 2 rural districts of Bangladesh, 22% of 2- to 3-y-old children consumed diets that were inadequate in zinc and an estimated 73–100% of women had inadequate zinc intakes. The prevalence of inadequate zinc intakes, low serum zinc concentrations, and stunting, all indicators of zinc deficiency, were higher in children residing in Trishal than in Pirgacha. Mean total zinc intakes of the women did not differ by district of residence; however, estimated absorbable zinc intakes were slightly and significantly lower in

TABLE 2 Serum zinc concentrations of children in 2 rural districts of Bangladesh[1]

	Both districts	Trishal	Pirgacha	*P*-value[2]
n	279	143	136	
Serum zinc, *μmol/L*				
Uncorrected	11.2 ± 1.9	10.4 ± 1.6	12.0 ± 1.8	<0.0001
Corrected for infection[3]	11.3 ± 1.9	10.6 ± 1.6	12.1 ± 1.8	<0.0001
Low serum zinc (<9.9 μmol/L),[3] *%*	24	36	11	<0.0001

[1] Data are mean ± SD.

[2] *P*-values are for district differences.

[3] Serum zinc values were adjusted using the β-coefficient for indictors of infection (4-level variable indicating high or low AGP and CRP) from a linear regression (PROC SURVEYREG) model that included as a covariate the time of day of blood sampling (defined as number of hours since midnight).

Pirgacha. Despite the low measured content of zinc in the rice from the study areas, rice contributed a large proportion of the daily zinc intakes of both women (69%) and children (49%). Simulated effects of increasing the zinc content of rice through biofortification suggest that considerable improvements in the total dietary zinc adequacy might be possible, depending on the level of adoption of biofortification in the communities, but effects on absorbable zinc appear to be lower.

Prior to the present survey, there was very little quantitative information on rice consumption by individuals among representative population samples in Bangladesh. The rice intakes of the women in our survey were similar to the mean 450 g/(person·d) reported in a small survey of 2 villages in Bangladesh, based on estimated intakes from household-level information (32). In our survey, children's rice intake tended to decrease over the pre-Aman harvest, post-Aman harvest, and Aus/Boro harvest seasons. The highest rice intakes were during the pre-Aman season in October–November among children in Trishal. In contrast, the lowest seasonal intakes by women occurred in the pre-Aman season. Interestingly, in another study conducted in a rural village in Bangladesh, pre-Aman harvest energy intakes were higher among young children, but lower among the mothers, compared with intakes during the post-harvest season, suggesting a possible shift in intra-household food distribution during periods of low rice availability (33). However, it is uncertain whether the seasonal trends we found in the present study were real or due to an imbalance in the numbers of subjects during the seasons surveyed, particularly the small number in the pre-Aman harvest season when data were collected only in Trishal.

The high prevalence of both low serum zinc concentrations and stunting in the children indicate a high risk of zinc deficiency, particularly in the Trishal district. Our findings are in agreement with other reports. In 3- to 7-y old Bangladeshi children, 50% had serum zinc concentrations < 9.9 μmol/L after adjusting for infection (10). In a national survey, 53–54% of 24- to 47-mo-old children were stunted (9).

It is uncertain whether the relatively large differences in serum zinc concentrations and stunting rates between the 2 districts can be attributed to differences in zinc intakes. Children in Pirgacha had only slightly, although significantly, higher total zinc intakes and zinc from animal sources. However, phytate intakes and phytate:zinc ratios were also higher in Pirgacha. Rice was the primary source of phytate, but the phytate

TABLE 3 Usual intake distributions of rice, energy, zinc, and phytate of young children and their primary female caregivers in 2 rural districts of Bangladesh[1]

	Both districts			Trishal			Pirgacha			
	n	Mean	Median (25th, 75th)	*n*	Mean	Median (25th, 75th)	*n*	Mean	Median (25th, 75th)	*P*-value[2]
Children										
Rice, raw, *g/d*	463	138	134 (99, 172)	226	148	145 (112, 180)	237	130	125 (90, 164)	0.11
Energy,[3] *kcal/d*	463	889	883 (756, 1015)	226	856	854 (736, 971)	237	922	915 (782, 1053)	<0.0001
Zinc, *mg/d*	463	2.5	2.5 (2.1, 2.9)	226	2.5	2.4 (2.0, 2.9)	237	2.6	2.5 (2.1, 3.0)	0.005
Zinc from animal sources, *mg/d*	463	0.61	0.52 (0.32, 0.80)	226	0.46	0.39 (0.23, 0.61)	237	0.75	0.67 (0.46, 0.95)	<0.0001
Phytate, *mg/d*	463	286	272 (213, 344)	226	260	253 (204, 307)	237	312	295 (229, 375)	<0.0001
Phytate:zinc molar ratio	463	11.4	11.2 (9.8, 12.8)	226	10.6	10.5 (9.6, 11.5)	237	12.1	12.0 (10.3, 13.8)	0.0001
Primary female caregivers										
Rice, raw, *g/d*	478	422	420 (365, 476)	238	401	398 (347, 451)	240	443	443 (387, 498)	0.002
Energy,[3] *kcal/d*	478	1883	1869 (1608, 2142)	238	1756	1757 (1516, 1975)	240	2005	2004 (1760, 2244)	<0.0001
Zinc, *mg/d*	478	5.5	5.4 (4.8, 6.1)	238	5.5	5.4 (4.7, 6.2)	240	5.5	5.4 (4.8, 6.1)	0.87
Zinc from animal sources, *mg/d*	478	0.63	0.54 (0.35, 0.80)	238	0.52	0.43 (0.26, 0.66)	240	0.75	0.71 (0.55, 0.90)	<0.0001
Phytate, *mg/d*	478	656	643 (551, 746)	238	586	578 (500, 663)	240	725	712 (613, 821)	<0.0001
Phytate:zinc molar ratio	478	12.0	12.0 (11.1, 12.9)	238	10.8	10.8 (10.2, 11.4)	240	13.3	13.3 (12.6, 14.0)	<0.0001
Absorbable zinc,[4] *mg/d*	478	1.3	1.3 (1.2, 1.3)	238	1.3	1.3 (1.2, 1.4)	240	1.2	1.2 (1.2, 1.2)	<0.0001

[1] Values are unadjusted means, medians (25th, 75th percentile).

[2] The *P*-values are for district differences in rice or nutrient intake in multivariate models that included covariates for season (children and women models), age group (children model only), and breast-feeding status (children model only).

[3] 1 kcal = 4.184 kJ.

[4] Absorbed zinc was estimated using prediction equation by Miller et al. (31).

TABLE 4 Prevalence of inadequate zinc intakes and simulated impact of biofortified rice on zinc intake inadequacy in young children and their primary female caregivers in 2 rural districts of Bangladesh

	Simulated population adoption levels[1]			
	Baseline	15%	35%	70%
Prevalence of inadequate zinc intakes of children[2]				
Both districts	21.9	19.2	15.3	8.9
Trishal	25.2	21.9	17.0	9.4
Pirgacha	18.0	16.1	13.4	7.9
Prevalence of inadequate zinc intakes of female caregivers				
Total zinc assuming higher bioavailability[3]				
Both districts	73.1	54.5	40.5	20.4
Trishal	70.3	55.3	42.1	23.5
Pirgacha	72.2	53.2	39.2	18.9
Total zinc assuming lower bioavailability[4]				
Both districts	94.4	77.8	61.6	37.0
Trishal	90.6	77.3	63.3	39.7
Pirgacha	93.7	76.1	59.3	35.1
Absorbed zinc[5]				
Both districts	100	99.3	94.8	85.1
Trishal	100	98.4	91.2	76.0
Pirgacha	100	99.8	97.8	92.2

[1] Random samples of 15, 35, and 70% were selected to simulate different levels of adoption of zinc-biofortified rice in the population. For the simulations, an additional 8 μg/g (raw weight basis) of zinc was added to the district-specific baseline zinc rice concentrations. The numbers of participants were: both districts, *n* = 463 children; 478 women; Trishal, *n* = 226 children, 238 women; and Pirgacha, *n* = 237 children, 240 women.

[2] The cutoff for total dietary zinc adequacy of children is 2 mg/d (1).

[3] The cutoff for total dietary zinc adequacy with the assumption of higher bioavailability of a mixed or refined vegetarian diet is 6 mg/d (1).

[4] The cutoff for total dietary zinc adequacy with the assumption of lower bioavailability of an unrefined, cereal-based diet is 7 mg/d (1).

[5] The cutoff for absorbed zinc is 1.86 mg/d (1).

concentrations of rice analyzed from samples taken from the homes of study households were much lower than values previously reported (14). There is insufficient data in the literature to predict zinc absorption in children consuming different levels of zinc and phytate, so we were not able to adjust the children's total zinc intakes for bioavailability. The mean energy intakes of the children were close to requirements (34) and may have possibly been growth limiting for some children, particularly in Trishal where energy intakes were lower.

We estimated absorbable zinc intakes of the women using a prediction equation that takes into account total zinc and phytate intakes (31). Based on these estimates, all of the women had inadequate absorbable zinc intakes and the fractional absorption of zinc was estimated to be ~25%. Even though the mean phytate:zinc ratio of the women's diets was relatively low, the low total zinc intakes of the women limited the amount of estimated absorbable zinc.

The majority of women in our survey also had inadequate total zinc intakes. We chose to present estimates at 2 levels of assumed bioavailability, because without calculating absorbable zinc intakes we could have assumed a higher level of absorbability based on the phytate:zinc ratio alone. Nevertheless, total zinc intakes of the women were low and there were high levels of inadequacy using either assumption. Total zinc intakes of the women in our survey were lower than those reported from other studies of lactating or nonlactating women in the US and developing countries (35). Other reports of zinc intakes of Bangladeshi women are scarce; however, in a sample of 446 pregnant women residing in an urban slum of Dhaka, the median zinc intake was 6.5 mg/d (36), which is slightly greater than we observed in rural areas, but still below the recommended 8–10 mg/d for pregnant women (1). In our study, the zinc inadequacy of the women's diets was much greater than the children's despite a similar zinc density of ~2.9 mg/1000 kcal (4184 kJ). This discrepancy could be due to low energy intakes among many of the women or differences in the dietary requirements used to assess adequacy that are based on estimates of zinc bioavailability.

The percentage of women with inadequate total zinc intakes decreased significantly with a simulated high adoption rate of zinc-biofortified rice; however, the effects on absorbable zinc intake adequacy were not as great. Simulated increases in the zinc content of rice resulted in smaller increases in adequacy of absorbable zinc intakes than total zinc intakes. The relation between increasing total zinc intake and absorbable zinc intake is not linear, because the fractional absorption of zinc decreases with increasing total dietary zinc intake.

Interestingly, the women in Trishal had greater improvements in absorbable zinc intake adequacy than women in Pirgacha, but the opposite was true with simulations for total zinc intake adequacy. Rice intakes were greater in the Pirgacha women and one might expect a greater reduction in inadequate zinc intakes with biofortification. However, the zinc content of rice was lower in Prigacha; thus, impacts on total zinc intake adequacy were similar to that in the Trishal women. However, the women in Pirgacha also had higher phytate intakes and thus a lower zinc bioavailability than the women in Trishal, and this accounted for the lesser impact when amount of absorbed zinc was used in the simulation.

The interpretation and generalization of dietary zinc adequacy estimates should be made with some caution, because they are based on assumptions about zinc requirements and the population-specific intake distribution. The absolute amounts of the zinc requirements and intakes, of children in particular, are very small; therefore, measurement precision is important, because small absolute differences in intake can result in large differences in estimates of dietary adequacy. The children in our study had mean intakes that were higher than the theoretical requirement of 2 mg/d and therefore increases in the zinc content of rice had moderate effects on adequacy, because most of the children already had adequate intakes. The International Zinc Nutrition Consultative Group EAR for 1- to 3-y-old children was the same (2 mg/d) for both levels of assumed bioavailability, presumably due to rounding to a whole number. If we had used a theoretical zinc requirement of 2.5 mg/d as suggested by the Institute of Medicine (37), 51% of the children would have had inadequate intakes.

Our study has some limitations. Although we weighed foods prepared and consumed by the women and children during a 12-h daytime period, a portion of their daily intakes were assessed by recall. The proportion of daily intakes obtained by recall was higher for the women (22% of energy) than the children (8%), because the women often ate their last meal of the day later than the children and after the field worker had left the home. However, compared on a meal basis, the estimated intakes of energy were similar between the recalled and observed meals. Also, we were not able to collect quantitative information on breast milk intakes during the evening, so our estimates of daily zinc intakes for breast-feeding children may not be as accurate as for nonbreast-feeding children. However, the percent of zinc intake contributed by breast milk was very low, so this would not have a major impact on estimated total zinc intakes. Also, we attempted to improve our estimate of zinc status using serum zinc concentrations by adjusting for elevated acute phase proteins, but use of single cutoffs to define elevated CRP or AGP may have resulted in some misclassification of children's serum zinc status.

The improvements in total dietary zinc adequacy with simulated increases in the zinc content of rice are encouraging. The percentage of children and women with total dietary zinc inadequacy decreased by over 50% with a simulated high adoption rate of zinc-biofortified rice. However, a substantial proportion of the women would still have inadequate intakes of total and absorbable zinc at this level. Certain population subgroups with very low zinc intakes would likely benefit from larger increases in the zinc content of rice. Increasing the zinc content of rice is a potentially sustainable approach to improve zinc intakes of rural populations in Bangladesh. Other approaches to increase zinc intakes, such as increasing dietary diversity through animal-source foods, are difficult if the rural poor do not have the resources to purchase expensive animal foods. The cost effectiveness of zinc-biofortified rice needs to be assessed. Future studies are planned to examine children's zinc absorption from zinc-biofortified rice and the effects of consuming zinc-biofortified rice on indicators of zinc status.

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