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Bioavailability of Zinc from Cooked Philippine Milled, Undermilled, and Brown Rice, as Assessed in Rats by Using Growth, Bone Zinc, and Zinc-65 Retention

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The nutritional bioavailability of zinc from cooked milled, undermilled, and brown Philippine rice (variety PSB Rc14) was evaluated in rats, comparing results based on weight gain, tibia zinc incorporation (slope ratio analyses), and zinc radiotracer retention. Milling reduced the phytic acid and mineral content of the rice, resulting in zinc concentrations of 16.5, 19.4, and 27.2 μg/g and phytate/zinc molar ratios of 4, 20, and 28 for milled, undermilled, and brown rice, respectively. Measured zinc bioavailability was similar whether using growth, bone zinc, or radioisotope retention as criteria, at approximately 92, 86, and 77% of zinc sulfate, for milled, undermilled, and brown rice, respectively. However, the higher percent bioavailability of the zinc after milling was insufficient to compensate for the lower zinc content. With respect to zinc, the nutritional value was inversely related to milling, providing approximately 15, 17, and 21 μg bioavailable zinc/g rice, respectively, for milled, undermilled and brown rice of this variety.

KEYWORDS: Zinc bioavailability; absorption; retention; slope ratio; zinc-65; radioisotope tracer; brown rice; white rice; milling; phytic acid; weight gain; growth; tibia zinc

INTRODUCTION

There have been several attempts to popularize brown rice in Southeast Asia in place of milled rice. Brown rice is richer in fat, vitamins, protein, and minerals than milled rice, but also richer in dietary fiber and phytate that may inhibit absorption of minerals such as iron and zinc. This is of concern because, according to the recent 1998 national nutrition survey, 31% of Filipinos suffer from iron deficiency anemia (*1*). In addition, calculations of the global food supply suggest that 71% of the population in Southeast Asia is at risk for low zinc intake (*2*).

No human data are available comparing zinc bioavailability from brown and milled rice in Filipino diets. In Filipino adults, zinc absorption from two 3-d-germinated brown rice-based weaning foods was determined from the fecal recovery of zinc radioisotope, in comparison to that of unabsorbed chromium radioisotope. Zinc absorption was 14.7 ± 1.2% from a germinated brown rice/mungbean (7:3) mixture, and 12.0 ± 1.0% from a germinated brown rice/cowpea (7:3) formulation (*3*).

Baseline data on rats were desired prior to human studies on Filipinos. Previous data in rats showed a much lower zinc bioavailability in cooked brown rice than in cooked milled rice: 46 vs 92% of zinc sulfate when based on weight gain, and 32 vs 69% when based on total femur zinc, respectively (*4*). Zinc balance in rats showed an apparent absorption of 14% from brown rice, 23% from undermilled rice, and 22% from milled rice; the resulting femur zinc concentrations were 171, 188, and 194 μg/g, respectively (*5*). On the basis of whole body scintillation counting of zinc-65 radiotracer, rats retained 56.4% of the zinc from brown rice, which resulted in a bioavailability of 84% relative to that of a zinc chloride control (*6*).

The bioavailability of zinc is affected negatively by the molar ratio of phytate to zinc (*7*). As estimated in a WHO report (*7*), diets with a phytate/zinc molar ratio greater than 15 have relatively poor zinc bioavailability (10−15%), those with a phytate/zinc ratio between 5 and 15 have medium zinc bioavailability (30−35%), and those with a ratio less than 5 have high (45−55%) zinc bioavailability. However, limited human data on zinc absorption from practical whole diets suggest a more moderate influence of the phytic acid, resulting in 26 and 33% zinc absorption with phytate/zinc molar ratios of 14 and 5, respectively (*8*), and 29% zinc absorption with a phytate/zinc molar ratio of 7 (*9*). Phytate/zinc molar ratios are reported as 4−11 for milled rice (*4*, *5*, *10*), 11 for undermilled rice (*5*), and 13−47 for brown rice (*3*−*6*, *10*).

In view of these considerations and developments, a collaborative study was undertaken by the Philippine Rice Research Institute (PhilRice) Los Baños with the USDA Grand Forks Human Nutrition Research Center to study the bioavailability

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Table 1. Properties of Milled, Undermilled, and Brown PSB Rc14 Rices[a]

property	milled rice	undermilled rice	brown rice
100-grain weight (g)	1.67 ± 0.02	1.72 ± 0.02	1.80 ± 0.03
degree of milling (%)	7.2	4.4	0
chromameter L (white)	75.9 ± 1.2	64.3 ± 1.2	60.8 ± 1.8
chromameter a (red)	–0.1 ± 0.2	1.7 ± 0.1	1.7 ± 0.1
chromameter b (yellow)	10.1 ± 0.3	14.0 ± 0.4	16.5 ± 0.1
crude protein (%)	7.28 ± 0.30	7.84 ± 0.17	8.14 ± 0.16
crude fat (%)	0.41 ± 0.02	1.14 ± 0.03	1.94 ± 0.04
crude ash (%)	0.25 ± 0.03	0.45 ± 0.03	0.93 ± 0.02
phytic acid (mg/g)	0.65 ± 0.04	3.84 ± 0.28	7.80 ± 0.11
phosphorus (μg/g)	660 ± 21	1602 ± 10	2845 ± 57
potassium (μg/g)	506 ± 26	1221 ± 20	2184 ± 79
magnesium (μg/g)	151 ± 8	611 ± 4	1082 ± 17
calcium (μg/g)	87 ± 4	104 ± 1	132 ± 4
manganese (μg/g)	5.3 ± 0.3	10.2 ± 0.1	17.7 ± 0.3
iron (μg/g)	5.0 ± 0.4	7.6 ± 0.2	11.9 ± 0.3
zinc (μg/g)	16.5 ± 0.7	19.4 ± 0.1	27.2 ± 0.1
copper (μg/g)	2.58 ± 0.19	2.86 ± 0.02	3.23 ± 0.01
phytate/zinc molar ratio	4	20	28

[a] Data are mean ± SD ($n = 2$ for color, protein, fat, and ash determinations, $n = 3$ for all others) based on uncooked rice (color analyses and crude protein, fat, and ash) or on cooked, freeze-dried rice (phytic acid and mineral analyses).

of zinc from cooked milled, undermilled, and brown rices of the representative Philippine variety PSB Rc14, using rats. An additional goal of the research was to compare zinc bioavailability measurements, relative to a zinc sulfate control, by the slope-ratio assay with weight gain and bone zinc as criteria (*11*–*14*), and by retention of zinc-65 extrinsically added to the diet (*6*, *15*).

MATERIALS AND METHODS

Rice. The intermediate-amylose rice variety PSB Rc14 with intermediate gelatinization temperature and with good eating quality was selected for the study. PSB Rc14 rough rice from the 1996 dry season crop of PhilRice was used. The rice was aged and dehulled in a Satake THU35 dehuller and milled in a Satake One Pass Mill SKD Form DCK L2 at the Agricultural Processing Engineering Technology Department of the College of Agricultural Engineering and Technology, University of the Philippines Los Baños. The degree of milling was measured on the basis of decrease in the 100-grain weight of rice, measured in triplicate. The Philippine standards for milled rice require 5–7% weight removal from brown rice based on bran removal. The samples were measured for color with the Minolta Chromameter model CR-110 in L (white), a (red), b (yellow) mode; crude protein content was determined by the Kjeldahl method (using the factor 5.95 g protein/g nitrogen); crude ash was measured by dry ashing; and crude fat was determined by extraction (*16*) with petroleum ether instead of diethyl ether (**Table 1**).

The samples were air-cleaned to remove adhering bran, and 1600-g lots were washed once with 2080 mL of tap water (water/rice ratio 1.3) in a 2-L capacity rice cooker. After the wash water was replaced with fresh tap water, the rice was cooked using an automatic cooking cycle, then left undisturbed for 10 min, cooled in a sealed plastic bag for 1 h, frozen, and freeze-dried. Brown rice was soaked for 30 min in water before cooking. The yields were 226, 220, and 219 g of cooked milled, undermilled, and brown rice per 100 g of uncooked rice, respectively. The freeze-dried samples were stored at −20 °C until they were ground into a coarse powder. A 6-kg portion of each rice was air-freighted to Grand Forks, ND, where they were stored at 0 °C until the rat assay. Samples of the freeze-dried cooked rices were analyzed for phytic acid (*17*), and for zinc and other elements as described below.

Animals. Seventy-eight male Sprague–Dawley rats (35–49 g; Sasco Labs, Omaha, NE) were housed individually in suspended stainless steel cages. A 12-h light–dark cycle was used, with light on at 9 pm. The animals were randomly assigned to 13 diet treatment groups of 6 animals each, blocking on body weight. The animals consumed the experimental diets ad libitum (except for the day of zinc-65 administration, described below) for 3.5 weeks. Body weight gain was assessed for all animals after 22 d. The rats were killed after days 22 and 23, and blood and tibia samples were taken for analyses. All experimental procedures conformed to the U.S. National Institute of Health, Public Health Service, and Animal Welfare Act guidelines for the ethical treatment of animals (*18*).

Rat Diets. For the slope ratio assays of zinc bioavailability, the rat diets were planned to provide similar graded quantities of rice, with a range of zinc-deficient intakes that would yield zinc-associated responses in animal growth or tibia zinc. AIN-93G diets (*19*) were modified to reduce zinc from the protein source by substituting egg white solids for casein. Because of the egg white substitution, additional biotin was added (2 mg total biotin/kg diet) and the mineral mix was modified as recommended by Reeves (*20*). Zinc was not included in the mineral mix. Zinc sulfate was added, as the source of zinc, to the diets of three treatment groups, resulting in final concentrations of 3.5, 6.3, and 9.6 μg Zn/g diet. For another nine dietary treatment groups, each of the three kinds of rice was added as the primary source of zinc, contributing 13, 26, and 39% rice by weight (**Table 2**), in substitution for starch. The ratio of cornstarch-to-dextrinized cornstarch was kept constant at 3:1, and no adjustments were made for the protein content of the rice. The thirteen experimental diets also included one prepared with no added zinc. Analyzed dietary zinc concentrations, which were similar, but not identical, to the planned dietary concentrations (**Table 2**), were used for bioavailability modeling.

Zinc-65 Retention. Retention of zinc-65 from the diets was determined with the rats that consumed the two highest concentrations of each of the four sources of zinc. Because food intake was being measured as a dependent variable, the same fasting/feeding schedule was used for all animals, whether or not they had zinc-65 administered. After the rats consumed the experimental diets ad libitum for 8 d, they were fasted for 23.5 h. Nine hours into this fast, at the beginning of the dark cycle, the animals consuming the diets with the two highest concentrations of each zinc source were provided with 1 g of their respective diets extrinsically labeled with 28 kBq (0.75 μCi) of zinc-65. These test meals were consumed within 1.5 h. The initial whole-body radiation dose was determined within 6 h by counting the animals in a custom-made small animal whole-body counter (*21*). All animals resumed their assigned diets ad libitum for the remainder of the study. Whole-body retention of zinc-65 was determined at 14, 16, 18, and 22 days, and was corrected for background, isotope decay, and any fluctuations in the measurement of a zinc-65 standard. At these relatively low dietary zinc concentrations, retention changed less than 2% between 14 and 22 days, so no correction was made for endogenous excretion of the isotope, and only day-14 retention data are reported.

Elemental Analyses. The different forms of rice, rat diets, and freeze-dried rat tibias were analyzed for zinc and other elements by destruction of organic materials with dry heat (400 °C) alternating with

Table 2. Rat Diet Composition,[a] with Related Food Intake, Weight Gain, Tibia Zinc Content, and Zinc-65 Retention Data Used to Assess Zinc Bioavailability

	no Zn	zinc sulfate			milled rice			undermilled rice			brown rice			pooled SD	p^b
rice in diet, %					13	26	39	13	26	39	13	26	39		
planned diet Zn, μg/g	0	3.5	7.0	10.5	2.1	4.3	6.4	2.5	5.0	7.6	3.5	7.1	10.6		
analyzed diet Zn, μg/g	0.6	3.5	6.3	9.6	2.7	4.8	7.0	3.1	5.6	7.8	4.1	7.5	11.0	0.2	
food intake, g/d	9	12	17	16	11	14	17	11	15	17	12	15	17	1	0.0001
zinc intake, μg/d	5	41	101	149	29	65	112	31	80	130	47	111	183	7	0.0001
body weight gain, g/d	0.7	3.1	6.3	6.0	2.3	4.4	6.1	2.1	4.8	6.2	2.8	5.4	6.2	0.4	0.0001
tibia Zn, μg	10	14	25	42	13	17	26	13	19	29	15	24	32	2.2	0.0001
zinc-65 2-wk retention, %			96	(95)		(96)	94		(93)	85		84	(74)	3	0.0001[c]

[a] AIN-93G diets (*19*) modified as described in the text. [b] The *p* value indicates the probability of differences related to dietary treatments, by ANOVA. [c] The ANOVA for zinc-65 2-wk retention compared only the treatments consuming 6–8 μg Zn/g diet; values in parentheses are provided for comparison, but were not included in the ANOVA.

nitric acid reflux. Following quantitative dilution of the ash with HCl and deionized water, the elements were detected by inductively coupled argon-plasma-emission spectrophotometry (model 1140, Jarrell Ash, Waltham, MA; **Table 1**). Analytical accuracy for the analyses of the rice, diet, and tibia samples was monitored by simultaneously assaying minerals in standard reference materials of apple leaves, corn kernel, and bovine liver (standard reference materials 1515, 8413, and 1577b from the U.S. National Institute of Standards and Technology), respectively.

Slope-Ratio Modeling and Statistical Analyses. Data on growth and total tibia zinc were analyzed by the slope-ratio assay method, expressing bioavailability relative to zinc sulfate (*22*). Total tibia zinc was transformed using the natural logarithm to linearize the response. For each set of criteria evaluated, linearity of the regression curves was ascertained for each source of zinc separately. Then, a single multiple-regression model was derived to determine the slope of the responses for all four dietary zinc sources, with the "no-added-zinc" group serving as the blank (*23*). Tests were conducted to determine whether the mean of the blank differed significantly from the common intercept for the four zinc sources. Confidence intervals for relative bioavailability were obtained by using Fieller's method (*22*). The effects of dietary treatment on other response variables were assessed by using one-way analysis of variance (*24*).

RESULTS

Rice Characteristics. PSB Rc14 rice was confirmed to have starch with intermediate amylose content and intermediate gelatinization temperature. The milled rice samples were confirmed to have no bran streaks on the rice surface. The Chromameter "L" (whiteness) values showed the progressive whiteness of the samples with milling (**Table 1**). By contrast, both "a" (red) and "b" (yellow) colors decreased with the degree of milling. Crude protein, crude fat, ash, and phytic acid content of the rices decreased with the degree of milling. Mineral elements also decreased with the degree of milling and the gradients for zinc and iron were similar to those reported earlier (*5*, *25*).

Bioavailability Based on Growth. As expected (*26*), severe zinc deficiency depressed appetite and food intake (**Table 2**). Accordingly, bioavailability was modeled separately using dietary zinc concentrations (μg/g) as well as the absolute amount of zinc consumed (μg/d; calculated from dietary zinc concentration and food intake) as independent variables, and 22-d weight gain, or total tibia zinc as the dependent variables. Body-weight gain was positively associated with dietary zinc from each source (**Table 2**). Requirements for growth were met, and body-weight gain was not linearly related to dietary zinc above 8 μg/g (**Figure 1**), so data for rats consuming the highest concentrations of zinc from zinc sulfate or brown rice were excluded from the models based on weight gain. The overall slope ratio models relating weight gain to dietary zinc concentration (μg/g) or to

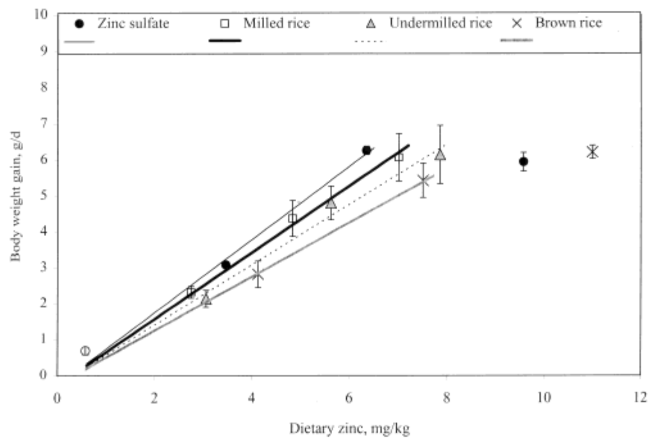

Figure 1. Mean (± SD) body-weight gain versus dietary zinc concentration, together with lines indicating the complete model, including all zinc sources, as further described in **Table 3**. Groups consuming more than 8 mg zinc/kg were excluded, because body weight did not respond linearly at these higher intakes.

absolute zinc intake (μg/d) explained 94 and 97% of the variability in the data, respectively. The R^2 for lines describing each zinc source exceeded 0.90 (**Table 3**; **Figure 1**; data based on absolute zinc intake are not graphed because of complexity). Zinc bioavailability from milled rice, undermilled rice, and brown rice, was 91, 83, and 75%, respectively (relative to zinc sulfate = 100%), based on dietary zinc concentration, and 90, 80, and 77%, respectively, based on absolute zinc intake (**Table 4**).

Bioavailability Based on Tibia Zinc. Tibia zinc data were logarithmically transformed to obtain a linear fit. The slope ratio models based on tibia zinc, like those based on weight gain, again explained over 90% of the variability in the data (**Table 3**; **Figure 2**). By using these models, relative zinc bioavailability from milled rice, undermilled rice, and brown rice, was 94, 91, and 76%, respectively, based on dietary zinc concentration, and 89, 85, and 72%, respectively, based on absolute zinc intake (**Table 4**). Although generally similar results were obtained whether using the tibia zinc or weight gain data to determine the relative zinc bioavailability, the difference between the milled and undermilled rice was less when using tibia zinc as the dependent variable (**Figure 2**; **Table 4**).

Bioavailability Based on Zinc-65 Retention. The whole-body retention of zinc-65 tended to decrease as the zinc concentration of the diet increased (**Table 2**). Thus, bioavailability comparisons were limited to the animals fed similar dietary concentrations of zinc from each of the zinc sources. Zinc-65 retention was 96, 94, 85, and 84% of the dose

Table 3. Slope-Ratio Models for Expressing Zinc Bioavailability from Growth and Bone Incorporation Data

independent variable[a]	criterion	model $R^{2\ b}$	model parameter/ source of zinc	regression coefficient[c] ± standard error	p value
dietary Zn, μg/g	weight gain, g	0.94	intercept	−0.266 ± 0.186	NS
			no added zinc	0.967 ± 0.263	0.001
		(0.98)	zinc sulfate	1.016 ± 0.043	0.0001
		(0.92)	milled rice	0.926 ± 0.040	0.0001
		(0.90)	undermilled rice	0.842 ± 0.035	0.0001
		(0.92)	brown rice	0.758 ± 0.037	0.0001
dietary Zn, μg/d	weight gain, g	0.97	intercept	1.047 ± 0.101	0.0001
			no added zinc	−0.346 ± 0.170	0.05
		(0.99)	zinc sulfate	0.051 ± 0.002	0.0001
		(0.95)	milled rice	0.046 ± 0.002	0.0001
		(0.93)	undermilled rice	0.041 ± 0.001	0.0001
		(0.98)	brown rice	0.039 ± 0.002	0.0001
dietary Zn, μg/g	ln(tibia Zn, μg)	0.94	intercept	2.11 ± 0.04	0.0001
			no added zinc	0.24 ± 0.06	0.0001
		(0.97)	zinc sulfate	0.17 ± 0.01	0.0001
		(0.90)	milled rice	0.16 ± 0.01	0.0001
		(0.90)	undermilled rice	0.15 ± 0.01	0.0001
		(0.89)	brown rice	0.13 ± 0.01	0.0001
dietary Zn, μg/d	ln(tibia Zn, μg)	0.95	intercept	2.3 ± 0.03	0.0001
			no added zinc	0.015 ± 0.048	NS
		(0.98)	zinc sulfate	0.0091 ± 0.0003	0.0001
		(0.91)	milled rice	0.0081 ± 0.0004	0.0001
		(0.90)	undermilled rice	0.0078 ± 0.0004	0.0001
		(0.92)	brown rice	0.0065 ± 0.0003	0.0001

[a] The independent variable, dietary zinc, was separately modeled by expressing this variable as the dietary concentration of zinc (μg/g) provided, as well as the amount of zinc consumed by the animals (μg/d). Although the latter variable accounts for the differences in food consumption associated with zinc deficiency, the resulting zinc bioavailability measurements were not substantially affected (see **Table 4** also). [b] The R^2 for the complete model is listed first, on the same line as the *X* and *Y* variables. Separate R^2 values, indicated in parentheses, are provided for simple linear models that included data from only one source of zinc (the simple linear models for zinc sulfate included the "no-added-zinc" group). [c] The regression coefficient is the slope for each source of zinc.

Table 4. Zinc Bioavailability of Milled, Undermilled, and Brown Rice Fed to Rats, as Measured by Five Criteria

	zinc sulfate	milled rice	undermilled rice	brown rice	milled rice	undermilled rice	brown rice
zinc in rice, μg/g					16.5	19.4	27.2
method/criteria	relative bioavailability, %				relative bioavailability, μg/g rice[a]		
growth vs diet zinc concentration	100	91 (87, 95)[b]	83 (80, 86)	75 (71, 79)	15	16	20
growth vs zinc consumed, μg/d	100	90 (85, 96)	80 (75, 86)	77 (71, 82)	15	16	21
tibia zinc vs diet zinc concentration	100	94 (88, 102)	91 (85, 97)	76 (71, 80)	16	18	21
tibia zinc vs zinc consumed, μg/d	100	89 (81, 97)	85 (79, 92)	72 (66, 77)	15	16	20
zinc-65 retention @ 6–8 μg zinc/g diet	100	98 (95, 101)	89 (86, 92)	87 (84, 90)	16	17	24
zinc-65 retention @ 9–11 μg zinc/g diet[c]	100			78 (73, 83)			21
average of 5 methods/criteria	100	92	86	77	15	17	21

[a] Product of % bioavailability relative to zinc sulfate multiplied by the concentration of zinc in the rice, and divided by 100. This somewhat arbitrary calculation to facilitate comparisons between the rices assumes 100% bioavailability of the zinc from zinc sulfate, which overestimates the actual zinc-65 retention data (**Table 2**) by 4–5%. [b] 95% confidence intervals in parentheses. [c] Not included in average of 5 methods, because data were not available for the other kinds of rice.

administered from diets containing 6.3, 7.0, 7.8, and 7.5 μg/g zinc as zinc sulfate, milled rice, undermilled rice, and brown rice, respectively (**Table 2**). Accordingly, zinc bioavailability relative to zinc sulfate was 98, 89, and 87% from milled rice, undermilled rice, and brown rice, respectively, based on 2-wk zinc-65 retention (**Table 4**). However, it is also notable that the bioavailability of zinc from brown rice, relative to zinc sulfate, was considerably lower, 78%, when tested at zinc concentrations of 9−11 μg Zn/g diet, than when tested at zinc concentrations of 6−8 μg Zn/g diet (**Table 4**).

Summary of Zinc Bioavailability Measurements. On the basis of an arbitrary average of the five methods and criteria used to assess the relative bioavailability of zinc from these rices with different degrees of milling, zinc from milled, undermilled, and brown rice was 92, 86, and 77% as bioavailable as from zinc sulfate (**Table 4**). However, the reduction in the zinc content of the rice had a greater nutritional impact than the associated increase in percent zinc bioavailability. Multiplying the percent bioavailability by the concentration of zinc in the rice allows comparison of the relative amounts of bioavailable zinc from the three kinds of rice (**Table 4**). Accordingly, the brown rice provided zinc that was the least bioavailable (77%) when compared across similar zinc intakes; however, it contained substantially more zinc (27.2 μg/g) (**Table 4**). Evaluated at comparable rice intakes, rather than comparable zinc intakes, brown rice provided considerably more bioavailable zinc (21 μg/g rice) than the milled or undermilled rices (15 and 17 μg/g rice, respectively). In support of this, when fed at the highest level of 39% of the diet, the brown rice as a source of zinc produced similar weight gain and slightly greater tibia zinc incorporation than the other two kinds of rice (**Table 2**).

DISCUSSION

The relative zinc bioavailability measurements reported for brown rice in the present study are considerably greater than

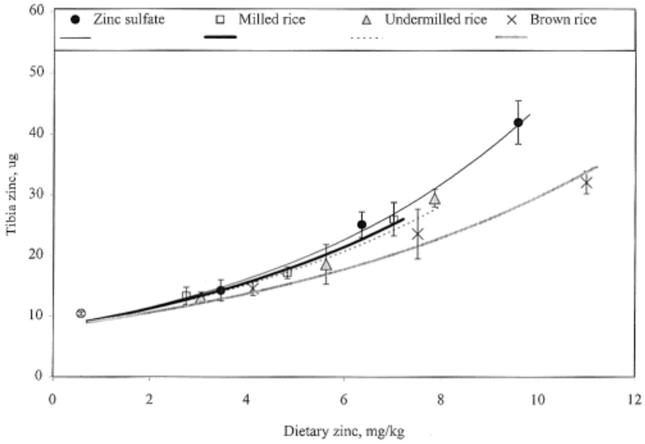

Figure 2. Mean (± SD) tibia zinc versus dietary zinc concentration, together with lines indicating the complete model, including all zinc sources, as is further described in **Table 3**. Although curved when shown on a linear scale, the relationships are linear when tibia zinc is logarithmically transformed.

those previously reported using a slope ratio method. Franz (*4*) reported relative zinc bioavailability values for cooked brown rice as 46 and 32%, based on weight gain and bone zinc, in contrast to 75−77 and 72−76%, respectively, in the present experiment (**Table 4**). Franz also reported relative zinc bioavailability values from cooked white rice of 92 and 69%, based on weight gain and bone zinc, in contrast to 90−91 and 89−94%, respectively, in the present experiment (**Table 4**). A reason for the greater bioavailability of the brown rice in the present experiment may be the lower phytic acid content of the rice. The brown and white rices reported by Franz had phytate/zinc molar ratios of 38 and 4, compared to 28 and 4 in the present experiment. Zinc bioavailability can also be influenced by the composition of the basal diet, especially the calcium content, and resulting 3-way interactions between calcium, phytic acid, and zinc (*27*). The diet used by Franz (*14*), compared to the AIN-93G diet of the present study, contained more calcium (6300 versus 5000 mg/kg diet) and had a higher phytate × Ca/Zn molar ratio (approximately 6 versus 3.5 mol/kg diet). This difference in the phytate × Ca/Zn molar ratio can substantially reduce the dietary zinc bioavailability, as measured using either weight gain or bone zinc as dependent variables (*27*). Therefore, bioavailability of zinc from the brown rice measured by Franz (*4*) was lower than that presently reported for the PSB Rc14 brown rice, because of both a higher phytic acid content of the rice and a higher calcium content of the basal diet.

The higher phytate content of the rice studied by Franz (*4*) may also explain why the weight gain and bone zinc criteria produced substantially different relative bioavailability results in that report, but not in the present study. Forbes (*13*) also reported considerably different results using weight gain, as compared with bone zinc, as criteria when testing foods that had low zinc bioavailability. Perhaps the relatively high zinc bioavailability of the brown rice in the present experiment resulted in greater similarity between the zinc bioavailability results based on weight gain and those based on bone zinc.

The zinc content of a food can be more important than the percent bioavailability. Because the differences in percent zinc bioavailability were greater than the differences in zinc content of the white and brown rices in the Franz report (*4*), the white rice in that report was a better source of absorbable zinc than the brown rice. The opposite conclusion must be drawn from the present data on the PSB Rc14 rice, commonly used in the Philippines (see relative bioavailability, μg/g rice, **Table 4**). This result emphasizes the usefulness of a separate evaluation of different rice varieties, as has been conducted in the present study. Rice-based diets may be improved through a knowledgeable selection of rice varieties and agronomic conditions, as well as milling practices that enhance the content of bioavailable minerals.

This study also provided an opportunity to compare methods of measuring relative zinc bioavailability in rats, by contrasting the slope-ratio method to a radiotracer retention method in the same animals. To evaluate zinc bioavailability by using the slope-ratio method, the animals had to be fed diets that did not meet their zinc requirements for growth or bone zinc incorporation. Evaluating radiotracer retention with zinc-deficient diets tended to increase the relative bioavailability measurement (**Table 4**). The bioavailability measurements using weight gain and tibia zinc criteria reflect all 22 d of inadequate dietary zinc; in contrast, the radiotracer bioavailability measurements reflect absorption after an initial 9 d of zinc deficient diet, allowing for partial gastrointestinal adaptation to increase zinc absorption. This could explain why the radiotracer bioavailability measurements were generally higher than those based on weight gain or tibia zinc (**Table 4**). A previous evaluation (*6*) of brown rice purchased in a U.S. retail market, by using the radiotracer method with animals fed 12 μg Zn/g diet, gave a relative zinc bioavailability result of 84%, which is comparable to the results of the present study. The radiotracer retention method was demonstrated to yield lower relative bioavailability values with rats fed diets with 35 μg Zn/g than rats fed diets with 12 μg Zn/g (*6*). We conclude that the radiotracer method is likely to produce results similar to those of the slope-ratio methods when the radiotracer retention is evaluated with diets that just exceed the zinc requirements for growth (approximately 10−12 μg Zn/g diet).

A less likely explanation for the greater bioavailability measurements using a radiotracer technique, compared to those based on weight gain or tibia zinc, could be a difference in the bioavailability of zinc intrinsic to the food vs zinc extrinsically added. The radiotracer retention method assumes that a tracer extrinsically added in the final stages of diet preparation forms a common pool with the dietary zinc prior to absorption, an assumption that has been generally (*28*−*30*) but not completely supported (*31*, *32*). In the present study, the similarities of the radiotracer results measured at 9−11 μg Zn/g diet (**Table 4**) to those based on growth or bone zinc (methods that do not rely on the extrinsic label assumption) support the conclusion that the extrinsic tracer and the zinc intrinsic to the rice were similarly available for absorption.

Although the slope ratio method relies on experimental diets that do not meet zinc requirements, the results suggest that by providing greater quantities of any of the three kinds of rice, the rats' zinc requirement could have been met. Assuming that the rats' requirement would be met if zinc sulfate provided 12 μg zinc per kg of diet (*26*), and dividing by the relative bioavailability data expressed as μg/g rice in **Table 4**, sufficient amounts of bioavailable zinc would have been provided with animal diets containing (by weight) approximately 80% milled, 71% undermilled, or 57% brown rice. Of course, in human diets it would be desirable to reduce these large proportions of rice by including other dietary sources of zinc such as meat, poultry, fish, beans, seeds, nuts, and other grains.

On the basis of the results of this study, attempts in Southeast Asia to popularize brown rice in place of milled rice in human diets may be expected to result in an increased amount of zinc absorbed. However, this conclusion may not hold if the phytate to zinc molar ratio of the brown rice exceeds that of the brown rice in the present report. The calcium content of the diet may also modify this conclusion; however, unless supplemented with large amounts of calcium salts, most human diets contain substantially less calcium, expressed in proportion to energy, than the animal diets used in either the present study or the study of Franz (*4*). Bioavailability results with animals may not be directly transferable to humans. In relation to this, it may be relevant that rats seem to have a greater intestinal phytase activity than humans (*33*). In addition, before making recommendations for human diets, the bioavailability of other nutrients, such as iron, from milled and brown rice should be considered. Although brown rice is a rich source of many nutrients, the advantages must be weighed against possible disadvantages of recommending brown rice such as consumer inconvenience because of longer cooking times, the reduced shelf life, and the increased possibility of contaminants such as mycotoxins, pesticides, or heavy metals in the outer layers of the grain.

In conclusion, an evaluation of a representative Philippine rice variety, PSB Rc14, indicated that milling decreased mineral content as well as phytic acid content of the rice. However, a resulting increase in the bioavailability of zinc was insufficient to compensate for the decrease in total zinc content of the milled or undermilled rice, and the brown rice remained a better source of zinc, as measured by rat growth, tibia zinc incorporation, and zinc radiotracer retention.

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