A Historical Review of Progress in the Assessment of Dietary Zinc Intake as an Indicator of Population Zinc Status[1–3]

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ABSTRACT

Dietary components influencing zinc (Zn) bioavailability were implicated in the first cases of human Zn deficiency in the Middle East in the 1960s. It was not until the 1980s that isotope tracer studies in humans quantified the effects of the type and/or quantity of Zn, protein, iron, and phytate (myo-inositol hexaphosphate) on Zn absorption in humans and confirmed the dose-dependent inhibitory effect of phytate on Zn absorption. This led to further analysis of the Zn and phytate content of foods. The use of phytate-to-Zn molar ratios as likely estimates of absorbable dietary Zn followed together with an assessment of their relationship with Zn biomarkers in low-income countries (LIC). In the 1990s, increasing knowledge of factors governing Zn-absorption diets led to refinements of Zn requirements and algorithms to estimate dietary Zn bioavailability. Their use highlighted that inadequate Zn intake from plant-based diets were a major etiological factor in morbidity and stunting in LIC, prompting the need to identify indicators of the population's Zn status. Major advances in analyses of dietary data pioneered by Beaton in 1980s led to the endorsement in 2007 of a dietary Zn indicator based on the prevalence of the population with usual Zn intake below the estimated average requirement for Zn. Risk of Zn deficiency is a public health concern when the prevalence of inadequate Zn intake is >25%. Recent findings that Zn bioavailability from high-phytate, whole-day diets is lower than previous estimates suggest that revision of Zn estimated average requirement for LIC may be warranted. *Adv. Nutr. 3: 772–782, 2012.*

Introduction

Zinc (Zn)[4] has been recognized as an essential nutrient for plants and animals since the 1920s and 1930s (1,2). In 1955, Tucker and Salmon (3) reported that parakeratosis in pigs was due to Zn deficiency. However, it was not until the early 1960s that human Zn deficiency was described in the Middle East, first in adolescent males in Iran by Prasad et al. (4) and then in Egypt (5,6). Some of the main clinical features observed were short stature and hypogonadism. Subsequent Zn supplementation studies among stunted prepubertal male farmers in Egypt (6) and stunted farmers in Iran (7) reported significant increases in height, weight, bone development, and secondary sexual maturation after treatment with Zn. In 1972, Hambidge et al. (8) documented Zn deficiency among young children in the United States who exhibited low growth percentiles, anorexia, hypogeusia, and low hair Zn concentrations. After treatment with Zn, taste acuity improved and hair Zn concentrations increased. These findings prompted a review on Zn nutrition in the United States by Sandstead (9), followed by further studies by Hambidge et al. in 1976. Male infants fed a Zn-supplemented cow's milk–based formula had greater gains in length and weight at 6 mo of age ($P < 0.05$) than either their female counterparts or control infants (10). Low-income Mexican-American preschool children were also reported to be at risk of Zn deficiency at this time (11).

Today, Zn is known to be involved in the activity of >300 enzymes in most major metabolic pathways and as an integral component of Zn finger proteins that regulate DNA transcription (12). Hence, Zn is necessary for a wide range of biochemical, immunological, and clinical functions. As a result, multiple functions in the body are affected by Zn deficiency, including physical growth, immune competence, reproductive function, and neurobehavioral development (13).

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[3] Supplemental Tables 1–3 are available with the online posting of this paper at advances.nutrition.org.

[4] Abbreviations used: IOM, Institute of Medicine; IZiNCG, International Zinc Nutrition Consultative Group; LIC, low-income country; Zn, zinc.

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There is no functional reserve or body store for Zn, so an adequate supply of dietary Zn is required on a regular basis. However, in many low income countries (LIC), staple diets are predominantly plant based; intake of cellular animal–protein foods such as red meat, poultry, and fish, rich sources of readily available dietary Zn, is often small because of economic, cultural, or religious constraints. As a result, the amount and/or bioavailability of Zn from such diets are low and frequently the primary cause of Zn deficiency. Hence an assessment of the prevalence of inadequate intake of dietary Zn can provide important information on the risk of Zn deficiency in a population.

Assessment of Zn levels in diets and the food supply

The first reports on the Zn content of diets were in the 1960s and 1970s and were based on the analysis of Zn in individual foods (14–16) or composite diets from a variety of settings (17–23). Foods of animal origin, especially oysters, meat (beef, pork, veal, lamb) and some seafood, nuts, seeds, dried legumes, and whole-grain cereals were good sources of dietary Zn; vegetables, fruits, and starchy roots and tubers had a lower Zn content, as shown in **Table 1**. In 1971, losses of Zn through food processing and preservation were quantified (24) and showed that milling decreased the Zn content of cereals; the Zn content of white bread was 77% lower compared with that of whole-grain bread because of the removal of the outer Zn-rich layers of the wheat grain.

The first national estimates of the Zn content of U.S. diets (13.3 mg/d) appeared in 1976, when Zn was included for the first time in the analysis of the Total Diet Study of the US FDA based on the market basket technique (20). However,

Table 1. Concentrations of zinc and phytate and phytate-to-zinc (Phy:Zn) molar ratios of selected foods

Food and scientific name	Zinc	Phytate	Phy:Zn molar ratio
Cereals[1]		*mg/100 g*	
Maize flour, 95% extraction (*Zea mays L.*)	2.2	792	36
Maize flour, 65% extraction	0.9	211	23
Maize bran	3.7	1089	29
Sorghum flour (*Sorghum bicolor L. Moench)*	1.4	446	32
Rice, white, raw (*Oryza sativa*)	1.8	100	5
Legumes[1]			
Ground nuts, boiled (*Arachis hypogaea L.*)	1.4	505	35
Ground nuts, flour	2.8	1297	45
Pigeon peas, fresh (*Cajanus cajan L. Millsp.*)	9.9	255	27
Pigeon peas, dry	2.2	727	33
Kidney beans, fresh (*Phaseolus vulgaris L.*)	1.5	557	36
Cow peas, boiled (*Vigna unguiculata L, Walp.*)	1.0	349	37
Lima beans, fresh (*Phaseolus lunatus L.*)	1.5	238	16
Bengal beans, fresh (*Stizolobium aterrimum Piper & Tracey*)	1.0	166	17
Vegetables (boiled)[1]			
Pumpkin leaf (*Cucurbita maxima Duch, ex* Lam.)	0.7	34	5
Chinese cabbage (*Brassico chinensis L.*)	0.7	5	1
Okra leaf (*Hibiscus esculentus* L.)	1.8	97	5
Cassava leaf (*Manihot esculenta Crantz*)	1.2	42	3
Cocoyam leaves (*Xanthosoma* sp. *Schott*)	0.6	19	3
Roots and plantain (boiled)[1]			
Sweet potato (*Ipomoea batatas L.*)	0.2	10	5
Yam (*Dioscorea sp. L*)	0.3	50	13
Cocoyam (*Xanthosoma* sp.)	0.5	37	7
Cassava (*Manihot* sp.)	0.3	54	18
Plantain, ripe (*Musa paradisiaca L.*)	0.2	0	0
Water yam (*Dioscorea alata* L.)	0.2	26	16
Meat, poultry, and organ meats[2]			
Beef, top sirloin, separable lean only, broiled	5.1	—	—
Lamb, loin, lean only, boiled	3.5	—	—
Pork, loin, bone-in, lean only, broiled	2.4	—	—
Chicken, breast, meat only, roasted	1.0	—	—
Chicken, thigh, meat only, roasted	2.6	—	—
Beef liver, pan fried	5.2	—	—
Fish and seafood[2]			
Flatfish (flounder and sole species) cooked, dry heat	0.6	—	—
Salmon, sockeye, cooked, dry heat	0.5	—	—
Crustaceans, crab, Alaska king, moist heat	7.6	—	—
Mollusks, oyster, wild, raw	90.8	—	—
Dairy products and eggs[2]			
Cow's milk, whole, 3.25% milk fat	0.4	—	—
Cheese, cheddar	3.1	—	—
Egg, whole, hard boiled	1.1	—	—

[1] Adapted from (25) with permission.
[2] Values from USDA (26).

it was not until 1980 that the analysis of Zn in ~300 U.S. foods was considered reliable enough to estimate the Zn levels (per capita per day) retrospectively in the U.S. food supply from food disappearance data (27). From 1909 until the early 1980s, the Zn content of the U.S. food supply was relatively stable, ranging from 13 to 11 mg per person per day in 1909 and 1980, respectively.

In 1982, Sandstead et al. (28) reported Zn intake from actual dietary intake of the US population. They calculated retrospective Zn intake from 24-h recalls collected in 3 earlier national surveys: the Nationwide Food Consumption Survey (1977–1978), the Ten State Nutrition Survey (1968–1970), and the Health and Nutrition Examination Survey II. From 1985, Zn intake was included in the Nationwide Food Consumption Survey (29) and continues to be reported at the national level today (30). In the US 1994–1998 Continuing Survey of Food Intakes by Individuals, the national estimate based on 24-h recalls was 10.4 mg/d (31). Many other industrialized countries (32–35) and some LIC (36–38) now report Zn intake based on national food consumption surveys.

Foods of animal origin were reported to be the major contributor of Zn in US diets from 1909 to 1980 based on food disappearance data (27), and the Total Diet surveys from 1975 to 1981/1982 (39). Animal foods continue to provide up to 50% of the Zn in adult diets in the United States (40), as well as other Western countries (32–34), although increasingly Zn-fortified cereals are becoming an important source of dietary Zn, especially among young US children (41,42), compared with those from other industrialized countries (43,44). Tracer studies have confirmed that the amount of Zn absorbed from high phytate-containing cereal products is greater from those fortified with Zn than when they are not fortified. Nevertheless, evidence showing a positive impact of Zn-fortified cereals on Zn-related health outcomes such as growth and morbidity in young children is sparse (45).

Dietary components influencing Zn absorption

Early animal studies indicated that Zn from plant-based foods was less available for intestinal absorption than that from animal sources (46,47). Several plant constituents were implicated, of which the most extensively studied in animal models at that time was phytate (myo-inositol hexaphosphate) (48–50). Human studies followed, based initially on metabolic balance studies that measured apparent Zn absorption (but not endogenous intestinal Zn losses) in subjects consuming a variety of plant and animal protein sources (51,52). Despite limitations in the study design and metabolic balance methods, results suggested that Zn absorption and retention was lower in persons consuming plant- versus animal-based diets. This finding stimulated extensive research using isotope tracers on the potential factors of plant-based diets affecting dietary Zn bioavailability in an effort to better estimate Zn requirements, initially with single test meals, but later with whole diets. These isotopic tracer studies were a significant advance because, unlike conventional metabolic balance studies, they could be designed to separate and measure endogenous Zn directly from unabsorbed dietary Zn in the feces, and thus measure "true absorption" without the use of a Zn-free diet. In 1989, Ziegler et al. (53) reported a lower amount of endogenous Zn excretion in infants fed a low Zn formula compared with those fed the regular formula. This finding led to the proposal that endogenous Zn excretion played a role in Zn homeostasis. Since this first report, significant progress has been made in understanding the critical role of endogenous fecal Zn excretion in Zn homeostasis by the pioneering stable-isotope studies of Hambidge and Krebs (54).

Phytate. Recognition in 1966 that intake of total Zn (i.e., 15 mg/d) derived from analysis of adult Egyptian village diets (17) appeared adequate compared with the provisional Zn requirements proposed at that time (12–15 mg/d) (55) led to the suggestion in 1967 that poor Zn bioavailability may have contributed to the pathogenesis of Zn deficiency in the Middle East (6). This suggestion prompted the analysis of phytic acid (myo-inositol hexaphosphate) concentrations of Iranian wheat flour flat breads using the analytical method of Oberleas (56) developed earlier. Results confirmed that the unleavened village bread, tanok, prepared from high-extraction wheat flour, had more phytate than the leavened city breads (630 vs. 301 mg/100 g) (57).

At that time, phytate was known to inhibit Zn absorption in animals (48,49), attributed to the formation of insoluble complexes in the gastrointestinal tract (58). Animal studies showed adverse effects of high phytate diets on Zn retention, Zn biomarkers, and weight gain, prompting the proposal to use phytate:Zn molar ratios to predict the bioavailability of Zn in phytate-containing diets (59,60). In 1978, Harland and Peterson (61) reported on phytate: Zn molar ratios in the vegetarian diets of Trappist monks, and 3 y later, Oberleas and Harland (62) proposed the use of phytate:Zn molar ratios to predict the likely Zn bioavailability in human diets.

Growing interest in the adverse effect of phytic acid on Zn absorption in humans prompted refinement of earlier phytate analytical methods and led to the accreditation by the Association of Official Agricultural Chemists of an anion-exchange method developed in 1986 (63). Compilations on the phytate and Zn content of foods and their corresponding phytate:Zn molar ratios followed (64–67). Seeds, nuts, legumes, and unrefined cereal grains were shown to have the highest phytate:Zn molar ratios, ranging from 17 to 45; other plant foods, notably vegetables, fruits, and tubers, had much lower ratios (i.e., 0–18) (Table 1). Even today, data on the phytate content of plant-based foods are limited and hence are not included in the food composition databases of most countries, including the USDA Nutrient Data for Standard Reference (26).

Following the first report by Harland and Peterson (61) on Trappist monks, several publications on the phytate:Zn molar ratios of diets of vegetarians and omnivores were reported in the late 1980s and early 1990s. These studies were based

initially on analysis of diet composites (68–70), but later on dietary intake calculated using food composition values for Zn and phytate from analyzed values and the published literature (71–78). Data for the diets of young children and adults are shown in Supplemental Tables 1–3. In some of these studies (69,72,75,77–79), the relationships between dietary phytate:Zn molar ratios, Zn biomarkers (e.g., serum or hair Zn), and Zn-related health outcomes such as stunting or impaired taste acuity were also examined. Millimolar ratios of phytate × calcium: Zn were also often included because at that time calcium was thought to accentuate the inhibitory effect of phytate on the bioavailability of Zn, based on studies in rats (58,80). Later, isotope studies in humans failed to confirm this finding in subjects consuming diets containing adequate levels of Zn (81), although whether high calcium levels adversely affect Zn bioavailability in individuals with a low Zn status is less clear.

Lönnerdal et al. (82) were among the first investigators to confirm the inhibitory effect of phytate on Zn absorption via a radioactive isotope study in infant rhesus monkeys and suckling rat pups and to demonstrate that the negative effect followed a dose-dependent response. This finding highlighted that Zn absorption could probably be improved in humans by reducing the phytate content of the diet. These same investigators demonstrated that only the higher inositol phosphates (i.e., hexa- and penta-inositol phosphates) inhibited Zn absorption; the lower inositol phosphates had no adverse effect. These findings were demonstrated first using a rat pup model (83) and later in a radioactive isotope study in humans (84). The latter human study was important because, unlike rats, hydrolysis of the higher inositol phosphates does not occur in the gastrointestinal tract of humans because of the absence of phytase enzymes (85). Lower inositol phosphates are produced during certain food preparation and processing methods such as fermentation (as occurred during leavening of city flat breads in Iran) and germination as a result of hydrolysis of phytate via microbial or endogenous phytase enzymes, respectively (86).

With the recognition of the importance of food preparation and processing methods on reducing the phytate content of foods came the development of newer techniques such as HPLC capable of separating and quantifying the individual inositol phosphates (87–89). Today, an HPLC method developed by Oberleas and Harland (90) is now the accredited Association of Official Agricultural Chemists method for phytate analysis, and the phytate content of selected foods analyzed by this method has been reported (91).

Dietary fiber. Dietary fiber was one of the plant constituents said to have the capacity to bind Zn and reduce its bioavailability based on early metabolic balance studies (51,52). However, because plant-based foods, particularly whole-grain cereals, nuts, and legumes, contain high levels of both dietary fiber and phytate, it was difficult to measure their independent effects using conventional balance studies.

With the advent of radioactive isotope studies in the 1980s, Navert et al. (92) showed that reducing the phytate content of bread via leavening had a positive impact on Zn absorption, leading to the suggestion that dietary fiber alone was not responsible for the adverse effect of plant-based foods on Zn absorption. At about the same time, Turnlund et al. (93) confirmed in a stable isotope study that Zn absorption was not affected by the insoluble fiber, α-cellulose, yet was markedly reduced by phytate.

Maillard browning products. Maillard reaction products, formed when foods containing a mixture of reducing sugars and amino acids are subjected to heat treatment, have the ability to complex metal ions, including Zn. In a radioactive isotope study, Zn absorption was lower from browned corn products (i.e., cornflakes) than from unbrowned corn products (i.e., corn grits), both intrinsically labeled with ^{65}Zn (94). Presumably Maillard reaction products bound Zn making it less available for absorption.

Iron. In the early 1980s, Solomons et al. (95,96) proposed the existence of an antagonistic interaction between nonheme iron and Zn. These findings were based on changes in plasma Zn uptake after the administration in the fasting state of aqueous solutions of inorganic Zn containing increasing amounts of ferrous sulfate as a source of nonheme iron. No comparable changes were observed with heme iron (95). However, these results were not confirmed in a later radioactive isotope study in which Zn absorption was measured when the same low (2.5:1) and high (25.0:1) ratios of nonheme iron and Zn were given with a meal, despite the apparent antagonistic effect when these same disproportionate molar ratios of nonheme iron to Zn were given as an aqueous solution (97). The absence of an inhibitory effect with food was presumed to be due to the chelation of nonheme iron and Zn to dietary ligands resulting from the digestion of food and thus absorbed by different pathways. This interaction could become important, however, if high doses of iron and Zn supplements are consumed without food.

Calcium. Earlier animal studies using rats (80,98,99) implied that calcium had a negative impact on Zn absorption, although only in the presence of high phytate-containing meals. The inhibitory effect was attributed to the formation of a calcium-Zn-phytate complex that was even more insoluble than either element combined separately with phytate. However, the adverse effect of calcium on Zn absorption was not confirmed in a later study based on whole body counting (81), as noted earlier. Calcium did not impair Zn absorption at adequate levels of Zn intake, irrespective of whether the dietary phytate content was low or high.

Quantity and quality of protein. As early as 1980, radioactive isotopes studies of Sandström et al. (100–102) showed that increasing the amount of total protein in a meal-enhanced Zn absorption, and if the protein was from cellular animal sources, the enhancing effect was even greater (103), attributed to the release of L-amino acid acids and cysteine-

containing peptides during digestion that form soluble ligands with Zn (101–103). Cow's milk, however, did not show this enhancing effect, attributed by some (104) to its high casein content and by others (105) to its high calcium content. Unlike cow's milk, the Zn content of human milk is highly bioavailable; reports of fractional Zn absorption range from 0.40 to 0.60, depending on the amount of Zn consumed in a single meal (106).

Animal proteins were also said to counteract the inhibitory effect of phytate on Zn absorption in radioactive isotope studies based on single meals (101). However, in these early studies, the phytate content of the meals supplied varied, limiting the interpretation of these results because phytate is such a strong determinant of Zn absorption. In a more recent radioactive isotope study in which phytate levels were controlled so they were equal in both vegetarian and meat diets, no difference was observed in the fractional absorption of Zn in the 2 whole-day diets (107). Nevertheless, the total amount of absorbed Zn was lower in the vegetarian diet because, as expected, it had a lower content of total Zn than did the meat diet.

Quantity and form of Zn in the diet. Changes in Zn absorption and gastrointestinal Zn secretion with increasing intake of dietary Zn were first studied in 1984 using stable isotopes by Jackson et al. (108). This was followed by a series of Zn stable isotope studies led by King et al. who studied Zn absorption in response to reductions in dietary Zn intake in adult men (109,110) and elderly subjects (111). In these studies, the Zn stable isotopes were divided among all the meals fed during a 24- or 48-h period; Zn absorption, intestinal excretion of endogenous Zn, and nonintestinal excretion of endogenous Zn via the kidneys, integument, semen, or menses were measured so that total endogenous losses could be estimated. These results were the first to indicate that Zn absorption was influenced by dietary Zn content, with homeostatic mechanisms up-regulating Zn absorption and retention, when intake of dietary Zn was low and vice versa. Later studies indicated that the up-regulation of Zn absorption may only be temporary. Instead, low endogenous Zn excretion via the intestine appears to play the major role in long-term whole body Zn homeostasis when dietary Zn intake are habitually low (54). Stable isotope studies in adults have shown a positive correlation between fecal excretion of endogenous Zn and absorption of exogenous dietary Zn (112).

Early studies suggested that absorption of Zn in the diet was also influenced by chemical form. Organic forms of Zn (in oysters) were said to be more readily absorbed than inorganic forms (113) and not affected by nonheme iron, as indicated by changes in plasma Zn uptake (95).

Classifying diets according to Zn bioavailability and developing dietary Zn requirements

Available data on dietary modifiers of Zn absorption led WHO in 1973 to classify diets into 3 categories according to whether the fractional absorption of dietary Zn (defined as available Zn) was 10%, 20%, or 40% available (114). Provisional estimates of dietary Zn requirements in relation to these estimates of availability were derived from physiological requirements compiled using the factorial approach by Sandstead (9). It was anticipated that comparison of Zn intake with a dietary requirement estimate that took into account the potential fractional absorption of Zn in habitual diets would provide a more valid assessment of the adequacy of dietary Zn intake. At that time, however, the data used for the requirement estimates were based on changes in lean tissue Zn concentrations with age and from crude metabolic balance studies in humans that measured apparent and not true Zn absorption because endogenous sources of fecal Zn were generally not measured.

In 1996, WHO published an extensive revision of the dietary Zn requirements (115) based on single-meal isotope tracer data published in the 1980s and 1990s and discussed earlier, which compared fractional Zn absorption from a variety of diet types, including some formula diets (101, 102,116). The revised system again used the factorial approach to estimate the average physiological requirements for absorbed Zn for certain age and physiological groups, which were then translated into requirements for Zn as ingested (i.e., dietary requirements), taking into account particular types of diets. WHO categorized diets into 3 broad categories of low, moderate, and high bioavailability, corresponding to revised estimates of ~15%, ~30% or 35%, and ~50% or 55% Zn bioavailability, respectively. For moderate and high bioavailability, 2 levels of dietary requirements were set, a basal and a normative requirement. The basal level reflected the ability of an individual to increase the efficiency of Zn retention when receiving restricted Zn intake, whereas the normative level applied to individuals who were consuming a Zn intake close to the requirements so that adaptation by increasing the efficiency of Zn retention was not necessary. The estimates for bioavailability took into account 3 major components of the diet: the phytate:Zn molar ratio, the amount of animal-source protein, and the amount of calcium, especially calcium salts. In the revision of the dietary Zn requirements by WHO/FAO in 2004, only the normative Zn requirements [μg/(kg of body weight$\cdot$d)] were specified so bioavailability estimates were 15%, 30%, and 50% (117).

In 2001, the Institute of Medicine (IOM) developed new estimates of physiological Zn requirements, again using the factorial approach (31). However, at that time, the data used to derive the estimates were from low-phytate or phytate-free meals. The inhibitory effect of dietary phytate on Zn absorption was recognized by the IOM committee, but the available data were deemed too limited to derive quantitative estimates of the effect of varying levels of phytate on the dietary Zn requirements. Instead, the IOM applied a factor of 40% for the bioavailability of Zn from mixed diets in the United States and Canada for adults (older than 19 y) and 30% for preadolescent children, based on data from studies of very low phytate intake. Indeed, the IOM committee cautioned that for strict vegetarians with diets with phytate:Zn molar ratios >15:1, the requirement for dietary Zn may be 50% greater (31). Certainly, more recent research has emphasized that the inhibitory effect of dietary phytate on Zn absorption is severe (118).

In 2004, the International Zinc Nutrition Consultative Group (IZiNCG) developed a new set of dietary Zn requirements for use in LIC (13), prompted in part by the more recent evidence that that the effect of dietary components on the efficacy of Zn absorption may have been overestimated in the earlier single test meal studies compared with the total diet studies. Hence, the data used by the IZiNCG were based only on total-diet tracer studies that represented typical diets consumed by populations irrespective of geographic location and that included studies on men and women; details of their derivation are given elsewhere (13,119).

the IZiNCG (13) classified diets into 2 diet types: mixed or refined vegetarian diets characterized by a phytate: Zn molar ratios of 4–18, and unrefined cereal-based diets with phytate: Zn molar ratios >18. For each diet type, a critical level of Zn absorption was derived for men and women by applying the prediction equation for the fraction of absorbable Zn based on phytate:Zn molar ratios of 11 and 24 for mixed and cereal-based diets, respectively, and the range of total Zn intake (i.e., 4.2 mg and 16.5 mg), which was used in the studies selected.

Currently, 3 algorithms exist to estimate intake of bioavailable Zn. The first algorithm developed by Murphy et al. (120) in 1992 was based on the semiquantitative classification system of WHO (115) for diets in LIC with a low content of animal protein, a moderate to low content of calcium, but a moderate to high content of phytate. In 2004, the IZiNCG developed a prediction equation to estimate bioavailable dietary Zn based on a regression model (13). Only Zn and the phytate:Zn molar ratio were included in the final model, and both were very significant predictors of the percentage of Zn absorption ($r^2 = 0.413$, $P < 0.001$). The stable isotope studies of Hambidge et al. that quantified the effect of different levels of phytate on both Zn absorption and intestinal excretion of endogenous Zn (54,112,121–123) led to the publication of a new physiologically based mathematical model of Zn absorption based on the quantities of dietary Zn and phytate. Modeling Zn absorption with this new trivariate model accounts for >80% of the variability in the quantity of Zn absorbed (124).

These 3 algorithms have been applied in several studies in LIC. In some cases, the interrelationships between intake of bioavailable Zn, Zn biomarkers, and/or Zn-related functional health outcomes such as linear growth were examined (120,125–129) For example, Murphy et al. (120,125) used their algorithm to calculate available Zn intake, first in toddlers and school-age children from rural villages in Egypt, Kenya, and Mexico who were participating in the Nutrition Collaborative Research Support Program study, and then later in an animal-source food intervention conducted on Kenyan school-age children (126). Estimates of available Zn intake based on this algorithm were also reported among infants and young children in rural Malawi (130,131). More recently, the IZiNCG regression model has been applied to estimate bioavailable Zn intake in studies in Mexico (127), whereas the trivariate model of Zn absorption of Miller et al. (124) was used in studies of Zn-biofortified wheat in Mexico (132), Zn-biofortified rice in Bangladesh (129), and in the calculations for the estimated minimum Zn fortification levels needed in wheat flour for adults (133). It is of interest that in the biofortified wheat study in adult women in Mexico, the absorbed Zn predicted by the trivariate model did not differ from measurements using the dual-isotope tracer ratio technique (132).

Development of a dietary indicator to assess risk of population Zn deficiency

In the past decade, results of meta-analyses identified Zn deficiency as a major factor in the etiology of diarrhea, acute lower respiratory infections, and impaired linear growth in LIC. Inadequate dietary Zn intake is a major factor associated with the development of Zn deficiency in these countries, making it important to use reliable methods to identify populations at high risk of inadequate Zn intake. Until recently, very few LIC have included an assessment of Zn status at the national level. In 2004, in an effort to provide countrywide estimates of the proportion of the population with inadequate Zn intake, Brown et al. (13) used food balance sheets in conjunction with the IZiNCG regression model to generate estimates of the mean per capita absorbable Zn content of national food supplies for comparison with the population's theoretical mean physiological requirement for Zn, which were updated in 2005 (134). Based on national estimates, regions and countries were ranked according to whether <15%, 15–25%, and >25% of the population were at risk of inadequate Zn intake due to inadequate Zn in the food supply. Overall, 20.5% of the world's population appeared to be at risk of inadequate Zn intake based on these estimates.

The national estimates generated from the food balance sheets emphasized the magnitude of the problem in LIC and the urgent need to collect nationwide food consumption data to assess the prevalence of inadequate Zn intake at the individual level. To assist countries in generating such data using the correct methods in their national nutrition surveys, the IZiNCG developed a technical brief in 2007 (135) for determining the risk of Zn deficiency based on dietary Zn intake.

Adjusting the distribution of observed intake to usual intake

The importance of within-person variability in dietary intake on the assessment of usual nutrient intake at the individual level was first highlighted by Beaton (136) in the late 1970s. He emphasized that repeated 24-h dietary recalls or records on nonconsecutive days should be collected so that the distribution of observed nutrient intake could be adjusted to usual intake by removing the variability introduced by within-person variation in nutrient intake. In 1986, a statistical method was developed by the US NRC to perform this adjustment (137), which was subsequently refined by Nusser et al. (138). In 2003, the IOM (139) suggested that in the absence of replicate observations from which to calculate within-person variance estimates for the population group under study, external variance estimates of within-person variation could be used, an approach that

was then adopted by Jahns et al. (140), and recommended by WHO/FAO in 2006 to formulate fortification levels (141) when replicate intake is not available.

Estimating the prevalence of inadequate intake from the distribution of usual Zn intake

As early as 1972, Beaton (142) pioneered the full probability approach to estimate the prevalence of inadequate intake, once the adjusted usual intake distribution was derived. The underlying assumptions of the model were described in 1986 in detail by the US NRC (137). The approach involves determining the probability of inadequacy of the usual nutrient intake level for each individual in the group, and then integrating these individual probabilities across the group to estimate the group prevalence. In 1992, Murphy et al. (120) were the first to use the full probability approach to estimate the prevalence of inadequate intake of Zn in toddlers and then in school-age children (125,126) in studies in Kenya, Egypt, and Mexico, followed by Ferguson et al. (73) in 1993 in a study of preschoolers in rural Malawi and Ghana.

In 1994, Beaton (143) proposed a short cut to the probability approach for assessing the proportion of inadequate intake in a group. This simpler version is termed the estimated average requirement (EAR) cut-point method and does not require information on the exact requirement distribution, although again data on the distribution of usual nutrient intake is required. In this simplified version, instead of estimating the risk of inadequacy for the intake level of each individual separately, the prevalence of inadequate intake within the group is simply estimated by counting the number of individuals in the group with usual intake below the estimated average requirements. Certain conditions must be met before the EAR cut-point method can be applied; these were discussed in detail by Carriquiry (144) and by the IOM (145). The adult national nutrition survey in New Zealand in collaboration with Beaton was the first national survey to apply both the statistical adjustment of Nusser et al. (138) and the EAR cut-point method to derive national prevalence estimates for inadequate intake of Zn (33,146). Since that time, these methods have been used in many other national nutrition surveys in developed countries (30,35) and LIC such as Mexico (127,147), Uganda (37), and Cameroon (38).

Validation of the dietary indicator

In 2007, Hotz (119) evaluated the validity of the dietary Zn indicator, developed by the IZiNCG and described in the technical brief (135). This was achieved by assessing the concordance among studies reporting the predicted risk of Zn deficiency based on both the IZiNCG dietary indicator and the prevalence of low serum Zn concentrations. Data from several small studies on adult nonpregnant and pregnant women, as well as from the most recent Mexican national nutrition survey (127), were examined. Data for adult women in the Mexican nutrition survey showed a reasonable concordance with the level of risk of Zn deficiency derived from the estimated prevalence of inadequate Zn intake and the prevalence of low serum Zn concentrations. Among studies in young children, however, there was less consistency between these 2 estimates, probably in part because of the limited data available to set both the EAR and cutoffs for low serum Zn concentrations for this age group; most of the data used were extrapolated from adults and older children, as discussed by Hambidge et al. (148) and Hotz (119). Nevertheless, based on these data, use of the dietary indicator described in the IZiNCG Technical Brief No. 3 was endorsed by WHO/UNICEF/International Atomic Energy Authority in 2007 (149).

More recent data based on the trivariant model generated by Hambidge et al. (118) in 2008 for adults suggest that the predicted inhibitory effect of dietary phytate on Zn absorption is much greater than previously estimated, unless there is some adaptation to habitual high-phytate intake. However, to date, there is no evidence that humans can adapt to habitual high-phytate intake by secretion of endogenous phytases (85). Furthermore, because recent Zn intake has a greater effect on fractional Zn absorption than long-term intake (150), up-regulation of Zn absorption when consuming habitual high-phytate intake is considered unlikely. As a result, the dietary requirement estimates for Zn may be higher than previous estimates for those individuals consuming predominantly high phytate plant-based diets in LIC. Indeed, application of the trivariate model by Hambidge et al. (118) suggested that the EAR for Zn appears to be doubled in adults when daily intake of phytate reaches 1000 mg/d. More experimental data are needed before similar predictions can be made for young children.

In 2011, Hambidge et al. (151) highlighted sources of discrepancy between the data and statistical methods used to derive estimates for the physiological requirements for Zn by the IOM (31) and Brown et al. (13). Clearly, these differences need to be resolved, and the physiological and dietary requirement estimates for Zn for use in LIC revised in light of these concerns. Attention must also be given to the increasing evidence that host-related factors such as tropical enteropathy may affect Zn homeostasis in children living in resource-poor households in LIC and hence must also be considered when setting dietary Zn requirements in these settings (152).

Applications of the dietary indicator

A dietary indicator is now recommended for national assessment of Zn status to indicate the need for Zn interventions and to measure changes in the adequacy of Zn intake in response to interventions designed to enhance Zn intake. The IZiNCG (135) defined the risk of Zn deficiency indicative of a public health concern as the prevalence of inadequate Zn intake >25%. To date, very few national surveys in LIC, with the exception of Mexico (127) and Cameroon (38), have used the IZiNCG dietary indicator to assess the prevalence of inadequate intake of Zn, although some recent smaller studies included this indicator (129). Nevertheless, several other national nutrition surveys in industrialized countries and LIC as well as some smaller studies have applied the steps recommended by the IZiNCG (135) to

generate prevalence estimates of inadequate Zn intake based on EAR more applicable to the dietary patterns of their own country (30,44) or the EAR set by WHO (120,126,131).

Additional uses for the dietary indicator have included the assessment of the potential changes in the prevalence of inadequate intake of Zn after the introduction of staple crops biofortified with Zn and Zn-fortified staple cereal flours. For example, Denova-Gutiérrez et al. (147) simulated the impact of substituting biofortified maize, wheat, and beans in the diets of women in Mexico on the prevalence of inadequate Zn intake, whereas Arsenault et al. (129) used the same approach to examine the potential impact of using biofortified rice in the diets of rural children and women in Bangladesh. Similarly, changes in the prevalence of inadequate Zn intake (and other micronutrients) in the presence and absence of wheat and/or maize flour fortified with Zn plus other micronutrients were also examined by Harvey et al. (37) in both children 24–59 mo of age and women of reproductive age in Kampala and Southwest and North Uganda. In the future, this same approach could be used to examine the impact of applying Zn fertilizers to Zn deficient soils to improve the Zn content of staple food crops (e.g., wheat, maize, sorghum, and beans) and, as a consequence Zn intake (153).

Conclusion and recommendations

In conclusion, major progress has been made in the development and application of a dietary indicator to assess the risk of population Zn deficiency. Nevertheless, many challenges still remain. It is hoped that more LIC will obtain data on dietary Zn intake, Zn biomarkers, and Zn-related functional indicators (e.g., stunting) in their national nutrition surveys in the future using the recommended techniques for the collection, statistical analyses, and interpretation of the dietary intake data. Additional measurements of fractional Zn absorption and intestinal endogenous Zn excretion based on stable isotope studies in young children consuming habitual diets with a wide range of Zn and phytate intake are also urgently required. Together, these data will provide much needed quantitative information on the magnitude of the effect of phytate on dietary Zn requirement estimates in young children so that the estimates for dietary Zn requirements in LIC can be refined. The level of conformity between the dietary, biochemical, and functional indicators of Zn status in national surveys should also be assessed to evaluate the validity of the current trigger levels set for identifying populations at high risk of Zn deficiency.

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Literature Cited

1. Sommer AL, Lipman CB. Evidence of indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1926;1:231–49.
2. Todd WR, Elvehjem CA, Hart E. Zinc in the nutrition of the rat. Am J Physiol. 1934;107:146–56.
3. Tucker HF, Salmon WD. Parakeratosis or zinc deficiency disease in the pig. Proc Soc Exp Biol Med. 1955;88:613–6.
4. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, hypogonadism, and geophagia. Am J Med. 1961;31:532–46.
5. Prasad AS, Miale A, Farid Z, Sandstead HH. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hypogonadism, and dwarfism. J Lab Clin Med. 1963;61:537–49.
6. Sandstead HH, Prasad AS, Schulert AR, Farid Z, Miale A, Bassilly S, Darby WJ. Human zinc deficiency, endocrine manifestations, and response to treatment. Am J Clin Nutr. 1967;20:422–42.
7. Halsted JA, Ronaghy H, Abadi P, Copper RL, Johnston KE, DuBard MB, Hauth JC. Zinc deficiency in man: the Shiraz experiment. Am J Med. 1972;53:277–84.
8. Hambidge KM, Hambidge C, Jacobs M, Baum JD. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. Pediatr Res. 1972;6:868–74.
9. Sandstead HH. Zinc nutrition in the United States. Am J Clin Nutr. 1973;26:1251–60.
10. Walravens PA, Hambidge KM. Growth of infants fed a zinc supplemented formula. Am J Clin Nutr. 1976;29:1114–21.
11. Hambidge KM, Walravens PA, Brown RM, Webster J, White S, Anthony M, Roth ML. Zinc nutrition of preschool children in the Denver Head Start program. Am J Clin Nutr. 1976;29:734–8.
12. Cousins RJ, McMahoh RJ. Integrative aspects of zinc transporters. J Nutr. 2000;130:1384S–7S.
13. Brown KH, Rivera JA, Bhutta ZA, Gibson RS, Hotz C, King JC, Lönnerdal B, Rivera JM, Ruel MT, Sandström B, Wasantwisut E. International Zinc Nutrition Consultative Group (IZiNCG) Technical Document # 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004;25: S94–203.
14. Murphy EW, Willis BW, Watts BK. Provisional tables on the zinc content of foods. J Am Diet Assoc. 1975;66:345–55.
15. Freeland JH, Cousins RJ. Zinc content of selected foods. J Am Diet Assoc. 1976;68:526–9.
16. Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. 4th edition. London: Her Majesty's Stationery Office; 1978.
17. Wallwork K, Koshakji RP. Content of zinc and iron in foods in Egyptian diets. First symposium in human nutrition and health in the Near East, May 1965. Lebanon: American University of Beirut; 1966.
18. White HS. Inorganic elements in weighed diets of girls and young women. J Am Diet Assoc. 1969;55:38.
19. Murphy EW, Page L, Watt BK. Trace minerals in type A school lunches. J Am Diet Assoc. 1971;58:115–22.
20. McGary ED, Young BE. Quantitative determination of zinc, iron, calcium, and phosphorus in the Total Diet market basket by atomic absorption spectrophotometry. J Agric Food Chem. 1976;24:539–42.
21. Guthrie BE, Robinson MF. Daily intake of manganese, copper, zinc, and cadmium by New Zealand women. Br J Nutr. 1977;38:55–63.
22. Abdulla M, Jagerstad M, Norden A, Qrist I, Svensson S. Dietary intake of electrolytes and trace elements in the elderly. Nutr Metab. 1977;21: suppl 1:41–4.
23. Holden JM, Wolf WR, Mertz W. Zinc and copper in self-selected diets. J Am Diet Assoc. 1979;75:23–8.
24. Schroeder HA. Losses of vitamins and minerals resulting from processing and preservation of foods. Am J Clin Nutr. 1971;24: 562–73.
25. Gibson RS, Ferguson EL. An interactive 24-h recall for assessing the adequacy of iron and zinc intakes in developing countries. HarvestPlus Technical Monograph 8. Washington, DC and Cali: International Food Policy Research Institute (IFPRI) and International Center for Tropical Agriculture (CIAT), 2008.
26. U.S. Department of Agriculture. USDA National Nutrient Database for Standard Reference, Release 16. Nutrient Data Laboratory, U.S. Government Printing Office, Washington, DC; 2011. http://www.nal.usda.gov/fnic/foodcomp

27. Welsh SO, Marston RM. Zinc levels of the U.S. food supply: 1909–1980. Food Technol. 1982;36:70–6.
28. Sandstead HH, Henriksen LK, Greger JL, Prasad AS, Good RA. Zinc nutriture in the elderly in relation to taste acuity, immune response, and wound healing. Am J Clin Nutr. 1982;36:1046–59.
29. Human Nutrition Information Service, Nutrition Monitoring Division. Nationwide Consumption Survey, continuing survey of food intakes by individuals: women 19–50 years and their children 1–5 years, 1 day. USDA Report No. 85-1;1985.
30. Alanna M, Goldman J, Cleveland L. What we eat in America, NHANES 2001–2002: usual nutrient intakes for food compared to dietary reference intakes. U.S. Department of Agriculture, Agricultural Research Service; 2005.
31. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academies Press; 2001.
32. McLennan W, Podger A. National nutrition survey selected highlights. 1995. Australian Bureau of Statistics, Canberra, Australia: Department of Health and Family Services; 1998.
33. Russell D, Parnell W, Wilson N, et al. NZ food: NZ people. Key results of the 1997 National Nutrition Survey. Wellington: Ministry of Health; 1999.
34. Department of Health. National Diet and Nutrition Survey. Headline results from years 1 and 2 (combined) of the rolling programme 2008/9–2009/10.Crown; 2011.
35. Parnell W, Scragg R, Wilson N, Schaaf D, Fitzgerald E. NZ food, NZ children: key results of the 2002 National Children's Nutrition Survey. Wellington: Ministry of Health; 2003.
36. Barquera S, Rivera JA, Safdie M, Flores M, Campos-Nonato I, Campirano F. Energy and nutrient intake in preschool and school age Mexican children: National Nutrition Survey 1999. Salud Publica Mex. 2003;45: Suppl 4:S540–50.
37. Harvey P, Rambeloson Z, Dary O. The 2008 Uganda Food Consumption Survey. Determining the dietary patterns of Ugandan women and children. USAID, A2Z, AED, Global Alliance for Improved Nutrition (GAIN), World Food Programme (WFP); 2010.
38. Engle-Stone R, Ndjebayi AO, Nankap M, Brown KH. Consumption of potentially fortifiable foods by women and young children varies by ecological zone and socio-economic status in Cameroon. J Nutr. 2012;142:555–65.
39. Pennington JAT, Wilson DB, Newell RF, Harland BF, Johnson RD, Vanderveen JE. Selected minerals in foods surveys, 1974 to 1981/82. J Am Diet Assoc. 1984;84:771–80.
40. Subar AF, Krebs-Smith SM, Cook A, Kahle LL. Dietary sources of nutrients among US adults. 1989 to 1991. J Am Diet Assoc. 1998;98:537–47.
41. Arsenault JE, Brown K. Zinc intakes of US preschool children exceeds new dietary reference intakes. Am J Clin Nutr. 2003;78:1011–7.
42. Briefel RR, Johnson CL. Secular trends in dietary intake in the United States. Annu Rev Nutr. 2004;24:401–31.
43. Hennessy-Priest KA, Mustard JL, Keller HH, Rysdale LA, Beyers JE, Goy R, Randall Simpson J. Zinc-fortified foods do not improve intake of total dietary zinc for Ontario preschoolers. J Am Coll Nutr. 2008; 27:561–8.
44. Gibson RS, Bailey KB, Parnell WR, Wilson N, Ferguson EL. Higher risk of zinc deficiency in New Zealand Pacific school children compared with their Maōri and European counterparts: a New Zealand national survey. Br J Nutr. 2011;105:436–46.
45. Hess SY, Brown KH. Impact of zinc fortification on zinc nutrition. Food Nutr Bull. 2009;30:S79–107.
46. O'Dell BL, Burpo CE, Savage JE. Evaluation of zinc availability in foodstuffs of plant and animal origin. J Nutr. 1972;102:653–60.
47. Davies NT. The effects of dietary fibre on mineral availability. Plant Foods. 1978;3:113–23.
48. O'Dell BL, Savage JE. Effects of phytic acid on zinc bioavailability. Proc Soc Exp Biol Med. 1960;103:304–6.
49. Oberleas D, Muhrer ME, O'Dell BL. Effects of phytic acid on zinc bioavailability and parakeratosis in swine. J Anim Sci. 1962;21:57–61.
50. O'Dell BL. Effect of dietary components upon zinc availability. Am J Clin Nutr. 1969;22:1315–22.
51. Reinhold JG, Faradji B, Abodi P, Ismail-Beigi F. Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fiber and phosphorus consumption as wheat bread. J Nutr. 1976;106:493–503.
52. Kelsay JL, Jacob RA, Prather ES. Effect of fiber from fruits and vegetables on metabolic responses of human subjects. III. Zinc, copper, and phosphorus balances. Am J Clin Nutr. 1979;32:2307–11.
53. Ziegler EE, Serfass RE, Nelson SE, Figueroa-Colón R, Edwards BB, Houk RS, Thompson JJ. Effect of low zinc intake on absorption and excretion of zinc by infants studied with ^{70}Zn as extrinsic tag. J Nutr. 1989;119:1647–53.
54. Hambidge M, Krebs NF. Interrelationships of key variables of human zinc homeostasis: relevance to dietary zinc requirements. Annu Rev Nutr. 2001;21:429–52.
55. Fox MR. Status of zinc in human nutrition. World Rev Nutr Diet. 1970;12:208–26.
56. Oberleas D. The determination of phytate and inositol phosphates. In: Glick D, editor. Methods of analysis. Vol. 20. New York: John Wiley & Sons; p. 87–101; 1971.
57. Reinhold JG. High phytate content of rural Iranian bread: a possible cause of human zinc deficiency. Am J Clin Nutr. 1971;24:1204–6.
58. Oberleas D, Muhrer ME, O'Dell BL. Dietary metal-complexing agents and zinc availability in the rat. J Nutr. 1966;90:56–62.
59. Davies NT, Olpin SE. Studies on the phytate:zinc molar contents in diets as a determinant of Zn availability to young rats. Br J Nutr. 1979;41:590–603.
60. Morris ER, Ellis R. Effect of dietary phytate:zinc molar ratio on growth and bone resorption of rats fed semipurified diets. J Nutr. 1980;110:1037–45.
61. Harland BF, Peterson M. Nutritional status of lacto-ovo vegetarian Trappist monks. J Am Diet Assoc. 1978;72:259–64.
62. Oberleas D, Harland B. Phytate content of foods: effect on dietary zinc bioavailability. J Am Diet Assoc. 1981;79:433–6.
63. Harland B, Oberleas D. Anion-exchange methods for determination of phytate in foods: collaborative study. J Assoc Off Anal Chem. 1986;69:667–70.
64. Oberleas D. Phytate content of foods. Cereal Foods World. 1983;28: 352–7.
65. Harland BF, Oberleas D. Phytate in foods. World Rev Nutr Diet. 1987; 52:235–59.
66. Le Francois P. Phytic acid and zinc contents of cereal products: relation to the manufacturing process. J Food Compost Anal. 1988;1: 139–45.
67. Reddy NR, Pierson MD, Sathe SK, Salunkhe DK. Phytates in cereals and legumes. Boca Raton: CRC Press; 1989.
68. Ellis R, Morris ER, Hill AD, Smith JC, Jr. Phytate: zinc molar ratio, mineral and fiber content of three hospital diets. J Am Diet Assoc. 1982;81:26–9.
69. Bindra GS, Gibson RS, Thompson LU. [Phytate][calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. Nutr Res. 1986;6:475–83.
70. Ellis R, Kelsay JL, Reynolds RD, Moser PB, Frazier CW. Phytate:zinc and phytate × calcium:zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese. J Am Diet Assoc. 1987;87: 1043–7.
71. Murphy SP, Calloway D. Nutrient intakes of women in NHANES II, emphasizing trace minerals, fibre, and phytate. J Am Diet Assoc. 1986;86:1366–72.
72. Ferguson EL, Gibson RS, Thompson LU, Oupuu S. Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. Am J Clin Nutr. 1989;50:1450–6.
73. Ferguson EL, Gibson RS, Opare-Obisaw C. The zinc nutriture of preschool children living in two African countries. J Nutr. 1993;123:1487–96.
74. Gibson RS, Heywood A, Yaman C. Solström A, Thompson LU, Heywood P. Growth in children from the Wosera subdistrict, Papua

New Guinea, in relation to energy and protein intakes and zinc status. Am J Clin Nutr. 1991;53:782–9.
75. Gibson RS, Smit Vanderkooy PD, Thompson L. Dietary phytate × calcium/zinc millimolar ratios and zinc nutriture in some Ontario preschool children. Biol Trace Elem Res. 1991;30:87–94.
76. Fitzgerald SL, Gibson RS, Quan de Serrano J, Portocarrero L, Vasquez A, de Zepeda E, Lopez-Palacious CY, Thompson LU, Stephen AM, Solomons NW. Trace element intakes and dietary phytate/Zn and Ca × phytate/Zn millimolar ratios of periurban Guatemalan women during the third trimester of pregnancy. Am J Clin Nutr. 1993;57:195–201.
77. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of peri-urban Guatemalan children in relation to zinc status: a cross-sectional study. Am J Clin Nutr. 1993;57:334–43.
78. Donovan UM, Gibson RS. The iron and zinc status of young women ages 14 to 19 years consuming vegetarian and omnivorous diets. J Am Coll Nutr. 1995;14:463–72.
79. Gibson RS, Huddle JMC. Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling and malaria. Am J Clin Nutr. 1998;67:702–9.
80. Davies NT, Carswell AJP, Mills CF. The effect of variation in dietary calcium intake on the phytate-zinc interaction in rats. In: Mills CF, Bremner I, Chesters JK, editors. Trace elements in man and animals. TEMA-5: CAB-Wallingford, UK, p. 456–7.
81. Hunt JR, Beiseigel JM. Dietary calcium does not exacerbate phytate inhibition of zinc absorption by women from conventional diets. Am J Clin Nutr. 2009;89:839–43.
82. Lönnerdal B, Bell JG, Hendrickx AG, Burns RA, Keen CL. Effect of phytate removal on zinc absorption from soy formula. Am J Clin Nutr. 1988;48:1301–6.
83. Lönnerdal B, Sandberg A-S. Sandström B, Kunz C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in sucking rats. J Nutr. 1989;119:211–4.
84. Sandström B, Sandberg A-S. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. J Trace Elem Electrolytes Health Dis. 1992;6:99–103.
85. Iqbal TH, Lewis KO, Cooper BT. Phytase activity in the human and rat small intestine. Gut. 1994;35:1233–6.
86. Gibson RS, Perlas LA, Hotz C. Improving the bioavailability of nutrients in plant foods at the household level. Proc Nutr Soc. 2006;65:160–8.
87. Graf E, Dintzis FR. Determination of phytic acid in foods by high-performance liquid chromatography. J Agric Food Chem. 1982;30:1094–7.
88. Lehrfeld J. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. Cereal Chem. 1989;66:510–5.
89. Lehrfeld J, Morris ER. Overestimation of phytic acid in foods by the AOAC anion-exchange method. J Agric Food Chem. 1992;40:2208–10.
90. Oberleas D, Harland B. Validation of a column liquid chromatographic method for phytate. J AOAC Int. 2007;90:1635–8.
91. Harland BF, Smikle-Willians S, Oberleas D. High performance liquid chromatography analysis of phytate (IP6) in selected foods. J Food Compost Anal. 2004;17:227–33.
92. Nävert B. Sandström B, Cedeblad A. Reduction of the phytate content of bran by leavening in bread and its effect on absorption of zinc in man. Br J Nutr. 1985;53:47–53.
93. Turnlund JR, King JC, Keyes WR, Gong B, Michel MD. A stable isotope study of zinc absorption in young men: effects of phytate and alpha-cellulose. Am J Clin Nutr. 1984;40:1071–7.
94. Lykken GI, Mahalko J, Johnson PE, Milne D, Sandstead HH, Garcia WJ, Dintzis FR, Inglett GE. Effect of browned and unbrowned corn products intrinsically labeled with ^{65}Zn on absorption of ^{65}Zn in humans. J Nutr. 1986;116:795–801.
95. Solomons NW, Jacob RA. Studies on the bioavailability of zinc in humans: effects of heme and nonheme iron on the absorption of zinc. Am J Clin Nutr. 1981;34:475–82.
96. Solomons NW, Pineda O, Viteri F, Sandstead HH. Studies on the bioavailability of zinc in humans: mechanism of the intestinal interaction of nonheme iron and zinc. J Nutr. 1983;113:337–49.
97. Sandström B, Davidsson L, Cederblad Å, Lönnerdal B. Oral iron, dietary ligands and zinc absorption. J Nutr. 1985;115:411–4.
98. Likuski HJA, Forbes RM. Mineral utilization in the rat. IV. Effects of calcium and phytic acid on the utilization of dietary zinc. J Nutr. 1965; 85:230–4.
99. Forbes RM, Erdman JW, Jr., Parker HM, Kondo H, Ketelson SM. Bioavailability of zinc in coagulated soy protein (tofu) to rats and effect of dietary calcium at a constant phytate:zinc ratio. J Nutr. 1983;113: 205–10.
100. Sandström B, Almgren A, Kivistö B, Cederblad Å, Bjorn-Rasmussen E. Zinc absorption from composite meals. 1. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. Am J Clin Nutr. 1980;33:739–45.
101. Sandström B, Cederblad Å. Zinc absorption from composite meals. II. Influence of the main protein source. Am J Clin Nutr. 1980;33: 1778–83.
102. Sandström B, Almgren A, Kivistö B, Cederblad Å. Effect of protein level and protein source on zinc absorption in humans. J Nutr. 1989;119:48–53.
103. Snedeker SM, Greger JL. Effects of dietary protein, sulfur amino acids and phosphorus on human trace element metabolism. J Nutr. 1983; 113:644–52.
104. Sandström B, Cederblad Å, Lönnerdal B. Zinc absorption from human milk, cow's milk and infant formulas. II. Influence of the main protein source. Am J Dis Child. 1983;137:726–9.
105. Spencer H, Kramer L, Norris C, Osis D. Effect of calcium and phosphorus on zinc metabolism in man. Am J Clin Nutr. 1984;40:1213–8.
106. Brown KH, Engle-Stone R, Krebs NF, Peerson JM. Dietary intervention strategies to enhance zinc nutrition. Promotion and support of breastfeeding for infants and young children. Food Nutr Bull. 2009; 30:S144–71.
107. Kristensen MB, Heks O, Morberg CM, Marving J, Bügel S, Tetens I. Total zinc absorption in young women, but not fractional zinc absorption, differs between vegetarian and meat-based diets with equal phytic acid content. Br J Nutr. 2006;95:963–7.
108. Jackson MJ, Jones DA, Edwards RH, Swainbank IG, Coleman M. Zinc homeostasis in man: studies using a new stable isotope-dilution technique. Br J Nutr. 1984;51:199–208.
109. Baer MT, King JC. Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am J Clin Nutr. 1984;39: 556–70.
110. Wada L, Turnlund JR, King JC. Zinc utilization in young men fed adequate and low zinc intakes. J Nutr. 1985;115:1345–54.
111. Turnlund JR, Michel MC, Keyes WR, King JC, Margen S. Use of enriched stable isotopes to determine zinc and iron absorption in elderly men. Am J Clin Nutr. 1982;35:1033–40.
112. Krebs NF, Miller LV, Naake VL, Sian L, Westcott JE. The use of stable isotope techniques to assess zinc metabolism. J Nutr Biochem. 1995;6: 292–307.
113. Solomons NW, Jacob RA, Pineda O, Viteri FE. Studies on the bioavailability of zinc in man: II. Absorption of zinc from organic and inorganic sources. J Lab Clin Med. 1979;94:335–43.
114. World Health Organization. Trace elements in human nutrition. Report of a WHO Expert Committee. World Health Org Tech Rep Ser. 1973;532:9–15.
115. World Health Organization. Trace elements in human nutrition and health. Geneva: World Health Organization;1996.
116. Sandström B, Almgren A, Kivistö B, Cederblad Å. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. J Nutr. 1989;117:1898–902.
117. World Health Organization/Food and Agriculture Organization. Vitamin and mineral requirements in human nutrition. 2nd ed. Geneva: World Health Organization; 2004.
118. Hambidge KM, Miller LV, Westcott JE, Krebs NF. Dietary reference intakes for zinc may require adjustment for phytate intake based upon model predictions. J Nutr. 2008;138:2363–6.
119. Hotz C. Dietary indicators for assessing the adequacy of population zinc intakes. Food Nutr Bull. 2007;28:S430–53.

120. Murphy SP, Beaton G, Calloway D. Estimated mineral intakes of toddlers: Predicted prevalence of inadequacy in village populations in Egypt, Kenya and Mexico. Am J Clin Nutr. 1992;56:565–72.
121. Adams CL, Hambidge KM, Raboy V, Dorsch JA, Sian L, Westcott JL, Krebs NF. Zinc absorption from a low phytic acid maize. Am J Clin Nutr. 2002;76:556–9.
122. Hambidge KM, Huffer JW, Raboy V, Grunwald GK, Westcott JL, Sian L, Miller LV, Dorsch JA, Krebs NF. Zinc absorption from low-phytate hybrid of maize and their wild-type isohybrids. Am J Clin Nutr. 2004; 79:1053–9.
123. Mazariegos M, Hambidge KM, Krebs NF, Westcott JE, Lei S, Grunwald GW, Campos R, Barahona B, Raboy V, Solomons NW. Zinc absorption in Guatemalan school children fed normal or low-phytate maize. Am J Clin Nutr. 2006;83:59–64.
124. Miller LV, Krebs NE, Hambidge KM. A mathematical mode of zinc absorption in humans as a function of dietary zinc and phytate. J Nutr. 2007;137:135–41.
125. Murphy SP, Calloway D, Beaton GH. Schoolchildren had similar predicted prevalence of inadequate intakes in toddlers in village populations in Egypt, Kenya and Mexico. Eur J Clin Nutr. 1995;49:647–57.
126. Murphy SP, Gewa C, Liang L-J, Grillenberger M, Bwibo NO, Neumann CG. School snacks containing animal source foods improve dietary quality for children in rural Kenya. J Nutr. 2003;133:3950S–6S.
127. Hotz C, Lowe NM, Araya M, Brown KH. Assessment of the trace element status of individuals and populations: the example of zinc and copper. J Nutr. 2003;133(5 Suppl):1563S–8S.
128. Abebe Y, Bogale A, Hambidge KM, Stoecker BJ, Arbide I, Teshome A, Krebs NF, Westcott JE, Bailey KB, Gibson RS. Inadequate intakes of dietary zinc among pregnant women from subsistence households in Sidama, Southern Ethiopia. Public Health Nutr. 2008;11:379–86.
129. Arsenault JE, Yakews EA, Hossain MB, Islam MM, Ahmed T, Hotz C, Rahman AS, Jamil KM, Brown KH. The current high prevalence of dietary zinc inadequacy among children and women in rural Bangladesh could be substantially ameliorated by zinc biofortification of rice. J Nutr. 2010;140:1683–90.
130. Hotz C, Gibson RS. Complementary feeding practices and dietary intakes from complementary foods amongst weanlings in rural Malawi. Eur J Clin Nutr. 2001;55:841–9.
131. Yeudall F, Gibson RS, Cullinan TR, Mtimuni B. Efficacy of a community-based dietary intervention to enhance micronutrient adequacy of high-phytate maize-based diets of rural Malawian children. Public Health Nutr. 2005;8:826–36.
132. Rosado JL, Hambidge KM, Miller LV, Garcia OP, Westcott J, Gonzalez K, Conde J, Hotz C, Pfeiffer W, Ortiz-Monasterio I, et al. The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification. J Nutr. 2009;139:1920–5.
133. Brown KH, Hambidge KM, Ranum P, Zinc Fortification Working Group. Zinc fortification of cereal flours: current recommendations and research needs. Food Nutr Bull. 2010;31 1 Suppl:S62–74.
134. Wuehler SE, Peerson JM, Brown KM. Use of a national food balance sheet data to estimate the adequacy of zinc in national food supplies: methodology and regional estimate. Public Health Nutr. 2005;8:812–9.
135. International Zinc Nutrition Collaborative Group. Determining the risk of zinc deficiency: assessment of dietary zinc intake. Technical Brief No. 03. IZiNCG, 2007.
136. Beaton GH, Milner J, Corey P, McGuire V, Cousins M, Stewart E, de Ramos M, Hewitt D, Grambsch PV, Kassim N, et al. Sources of variance in 24-hour dietary recall data implications for nutrition study, design, and interpretation. Am J Clin Nutr. 1979;32:2546–59.
137. National Research Council. Nutrient adequacy. Assessment using food consumption surveys. Washington, DC: National Academy Press; 1986.
138. Nusser S, Carriquiry A, Dodd K, Fuller W. A semi-parametric transformation approach to estimating usual daily intake distributions. J Am Stat Assoc. 1996;91:1440–9.
139. Institute of Medicine. Dietary reference intakes: applications in dietary planning. Washington, DC: National Academies Press; 2003.
140. Jahns L, Arab L, Carriquiry A, Popkin BM. The use of external within-person variance estimates to adjust nutrient intake distributions over time and across populations. Public Health Nutr. 2005;8:69–76.
141. World Health Organization/Food and Agriculture Organization. Guidelines on food fortification with micronutrients. Geneva: World Health Organization; 2006.
142. Beaton GH. The use of nutritional requirements and allowances. In: Proceedings of Western Hemisphere Nutrition Congress II. Mt. Kisco, NY: Futura; 1972. p. 356–63.
143. Beaton GH. Criteria of an adequate diet. In: Shils ME, Olson JA, Shile M, editors. Modern nutrition in health and disease, 8th ed. Philadelphia: Lea & Febiger; 1994. p. 1491–505.
144. Carriquiry AL. Assessing the prevalence of nutrient inadequacy. Public Health Nutr. 1999;2:23–33.
145. Institute of Medicine. Dietary reference intakes: applications in dietary assessment. Washington, DC: National Academies Press; 2000.
146. Gibson RS, McKenzie JE, Ferguson EL, Parnell WR, Wilson NC, Russell DG. The risk of inadequate zinc intake in United States and New Zealand adults. Nutr Today. 2003;38:63–70.
147. Denova-Gutiérrez E, Garcia-Guerra A, Flores-Aldana M, Rodriguez-Ramirez S, Hotz C. Simulation model of the impact of biofortification on the absorption of adequate amounts of zinc and iron among Mexican women and preschool children. Food Nutr Bull. 2008;29: 203–12.
148. Hambidge KM, Mazariegos M, Solomons NW, Westcott JE, Lei S, Raboy V, Grunwald G, Miller LV, Sheng X, Krebs NF. Intestinal excretion of endogenous zinc in Guatemalan school children. J Nutr. 2007;137:1747–9.
149. de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG Interagency meeting on zinc status indicators. Food Nutr Bull. 2007;28:S480–4.
150. Chung CS, Stookey J, Dare D, Welch R, Nguyen TQ, Roechl R, Peerson JM, King JC, Brown KH. Current dietary zinc intake has a greater effect on fractional absorption than does longer term zinc consumption in healthy adult men. Am J Clin Nutr. 2008;87:1224–9.
151. Hambidge KM, Miller LV, Westcott JE, Krebs NF. Physiological requirements for zinc. Int J Vitam Nutr Res. 2011;81:72–8.
152. Manary MJ, Abrams SA, Griffin IJ, Quimper MM, Shulman RJ, Hamzo MG, Chen Z, Maleta K, Manary MJ. Perturbed zinc homeostasis in rural 3–5-y-old Malawian children is associated with abnormalities in intestinal permeability attributed to tropical enteropathy. Pediatr Res. 2010;67: 671–5.
153. Cakmak I. Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J Trace Elem Med Biol. 2009;23:281–9.