Lead Article

Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies

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Plant-based diets in low-income countries (LICs) have a high content of phytic acid (myo-inositol hexaphosphate [InsP6]) and associated magnesium, potassium, and calcium salts. Together, InsP6 acid and its salts are termed "phytate" and are potent inhibitors of iron and zinc absorption. Traditional food processing can reduce the InsP6 content through loss of water-soluble phytate or through phytase hydrolysis to lower myo-inositol phosphate forms that no longer inhibit iron and zinc absorption. Hence, some processing practices can reduce the need for high-dose iron fortificants in plant-based diets and alleviate safety concerns. Dietary phytate-to-iron and phytate-to-zinc molar ratios are used to estimate iron and zinc bioavailability and to identify dietary iron and zinc requirements according to diet type. The European Food Safety Authority has set adult dietary zinc requirements for 4 levels of phytate intake, highlighting the urgent need for phytate food composition data. Such data will improve the ability to estimate the prevalence of inadequate zinc intakes in vulnerable groups in LICs, which will facilitate implementation of targeted policies to alleviate zinc deficiency.

INTRODUCTION

In many low-income countries (LICs), staple diets are often plant-based with unrefined cereals, legumes, and oleaginous seeds providing the major energy sources. These plant-based foods contain high levels of phytic acid (myo-inositol hexakisphosphoric acid [InsP6]) and associated magnesium, potassium, and calcium salts. Phytic acid and its salts are collectively termed phytate and are the principal storage form of phosphorus in mature seeds. In contrast, vegetables, fruits, starchy roots, and tubers are all low in phytate, and animal products have none.[1]

The phytate content of cereals, legumes, and oleaginous seeds varies widely, depending on the botanical variety, environmental or climatic growing conditions, use of phosphate fertilizers, and stage of maturation: the highest levels are reached at seed maturity.[1] In unrefined cereals, phytate is typically concentrated in the outer aleurone layer, except for maize, where it is mainly in the germ.[2] In legumes and most oilseeds, phytate is uniformly distributed within the protein bodies of the endosperm[3] or kernel.[4]

Phytic acid is made up of an inositol ring with 6 phosphate ester groups and is the most abundant form of myo-inositol phosphate found in mature, unprocessed, plant-based foods.[1] Phytic acid chelates cations, forming insoluble complexes with minerals in the upper gastrointestinal tract. These cannot be digested or absorbed by humans because of the absence of intestinal

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phytase enzymes.[5,6] During some food-processing and storage practices, however, InsP6 is dephosphorylated to lower myo-inositol phosphate forms, some of which no longer inhibit mineral absorption.[1,7]

Dietary phytate has been associated with certain health benefits, including blood glucose– and lipid-lowering effects, anticancer activity, antioxidant properties, and anticalcification.[1,7] The ability of phytate to bind toxic trace elements such as cadmium and lead and reduce their bioavailability may be an additional health benefit that warrants more extensive investigation; studies to date have yielded conflicting results.[1] In contrast, this review discusses the major adverse effect of phytate: its impact on mineral bioavailability. Food-processing practices that have the potential to reduce the phytate content of plant-based foods, thus minimizing the adverse effect on mineral bioavailability, are considered. Advantages and limitations of the analytical methods used for InsP6 and its lower phosphorylated forms are also addressed. Finally, implications of high-phytate diets for setting dietary requirements and formulating programs and policies to alleviate iron and zinc deficiencies in LICs are emphasized.

EFFECT OF PHYTATE ON BIOAVAILABILITY OF IRON AND ZINC IN HUMANS

Numerous investigations in animals[4,8] and in humans (mainly adults)[9–15] have demonstrated the inhibitory effect of phytate on absorption of minerals. Balance studies were conducted first,[8,9] followed by studies using radioactive[10,11] and stable isotope techniques.[12–15] Data from isotope techniques have highlighted that studies based on single meals exaggerate the inhibitory effect of phytate on absorption of iron[16] and probably zinc.[12,17] Of the minerals, fractional absorption of iron, zinc, calcium, and magnesium (but not copper) is substantially lower in high-phytate diets compared with diets with a low phytate content,[10,18–22] with absorption of iron and, to a lesser extent, zinc being most adversely affected.[20–24]

Adults have no adaptive response to increase zinc absorption[20,25] or to enhance reabsorption of endogenous zinc from habitual high-phytate diets,[26] although some adaptation with nonheme iron absorption has been found in long-term (ie, 10 wk)[27] but not short-term[28] studies. Whether zinc absorption in infants and young children is inhibited by high-phytate diets is less certain.[29]

In LICs, poor absorption from high-phytate diets is a major factor in the etiology of iron and zinc deficiency,[17,20,30] whereas for calcium deficiency, low intakes appear to be the most important factor.[13,14] Unlike calcium, magnesium intakes are likely to be high enough in unrefined cereal–based diets to largely outweigh any inhibitory effect of phytate on magnesium absorption.[15]

FOOD-PROCESSING PRACTICES WITH POTENTIAL TO REDUCE DIETARY PHYTATE

Several food-processing practices have the potential to reduce the phytate content of plant-based foods and diets (Table 1).[1,2,7,31–34]

Mechanical processing such as the milling of unrefined cereals can lead to marked reductions in phytate (and minerals), whereas dehulling legumes results in very little loss because phytate is evenly distributed in the endosperm of legumes.[3] Phytate is relatively heat stable during normal household cooking, except possibly phytate in tubers,[35] although there is some loss in canning and extrusion cooking when higher temperatures are used.[1]

Traditional household processing methods such as germination, fermentation, soaking, and hydrothermal processing may reduce the phytate content of unrefined cereals and legumes through dephosphorylation of InsP6 to lower myo-inositol phosphate forms, some of which no longer inhibit the absorption of zinc (InsP4–InsP1)[36] or iron (InsP2 and InsP1).[37] However, there is some evidence that in some processed foods even InsP3 and InsP4 may have a negative effect on zinc absorption.[38] Whether the inhibitory effect of phytate on calcium and magnesium absorption is restricted to higher myo-inositol phosphate forms (eg, InsP5 and InsP6) is uncertain.

Both intrinsic plant phytase enzymes and microbial phytases are capable of hydrolyzing InsP6. The extent of dephosphorylation depends on the plant species, the phytate content of the food, and the properties of the phytase enzymes, specifically their optimal temperature and pH.[7,31,39] For phytases in cereals, optimal activity occurs at 40°C–50°C and a pH around 5.0, whereas in legumes, pH optima vary, with pea having a pH optimum at 7–7.5.[31,39] Cereals, especially barley, rye, triticale, and wheat, and pseudo-cereals (buckwheat, amaranth) have a higher intrinsic phytase content than tropical cereals, such as maize and sorghum; legumes; and oil seeds.[31] During germination, phytase activity increases in most legumes and oil seeds and certain cereals, possibly through de novo synthesis, activation of intrinsic phytases, or both.[31] For example, after 72 hours of germination, substantial increases in phytase activity have been reported in lentils, mung bean, and pea, as well as maize, millet, and sorghum, with reductions in InsP6 being most pronounced for mung beans, rice, and millet.[40]

Table 1 **Influence of food-processing methods on phytate content of plant-based foods**

Processing method	Main technical influences and nutritional consequences	References
Milling cereals	Reduces phytate (and mineral) content in cereals when phytate is located in outer aleurone layer or in the germ (maize, millet, sorghum)	O'Dell et al (1972)[2]
Thermal processing	During household cooking, phytate is relatively heat stable. Modest losses of phytate occur with the high temperatures used in canning and extrusion cooking	Schlemmer et al (2009)[1]; Kumar et al (2010)[7]
Malting, followed by germination	Increases intrinsic phytase activity, which hydrolyzes phytate to lower inositol phosphate forms, some of which no longer inhibit iron or zinc absorption. Extent of hydrolysis depends on plant species, duration and conditions (pH, temperature, humidity) for optimal phytase activity	Schlemmer et al (2009)[1]; Kumar et al (2010)[7]; Greiner and Konietzny (2006)[32]
Fermentation	Microbial phytases hydrolyze phytic acid (InsP6) to lower inositol phosphate forms provided the pH, temperature, and humidity used are optimized for phytase. In addition, once the pH is reduced by the lactic fermentation of cereals, then the intrinsic cereal phytases become activated, degrading phytic acid further	Schlemmer et al (2009)[1]; Kumar et al (2010)[7]; Greiner and Konietzny (2006)[32]
Soaking in water, followed by decanting the water	Depending on plant species, temperature and pH, some diffusion of water soluble sodium and potassium phytates may occur, especially when cereals or legumes are not intact but in the form of flours or grits	Schlemmer et al (2009)[1]; Kumar et al (2010)[7]; Gibson et al (2006)[31]
	Also activates intrinsic phytases. Extent of phytase-induced hydrolysis depends on form of cereal or legume (ie, intact, flour, or grits) and optimal temperature, pH, and humidity for intrinsic phytase activity	Greiner and Konietzny (2006)[32]
Hydrothermal treatment	The treatment maximizes activation of intrinsic phytases and hence phytate degradation by providing optimal temperature and pH conditions for specific phytases at each step of the process. For some cereals, phytate reductions ranging from 84% to 99% have been achieved	Fredlund et al 1997[33]; Bergman et al 1999[34]

Fermentation is activated by phytase enzymes in microflora that occur naturally and/or are introduced via inoculation with exogenous microbial cultures. Organic acids (eg, lactic and acetic acids) produced during bacterial fermentation lower the pH, which favors the activation of both intrinsic cereal and exogenous microbial phytases.[1,7] Most microbial phytases are optimally active over a broad pH range, generally 3.5–6.0, with a temperature optimum from 35°C to 80°C.[31] Asian food products, such as tempeh, misa, koji, and soy sauce, are all prepared from soy beans fermented by microbial phytases from molds, specifically *Rhizopus oligosporus* and *Aspergillus oryzae*.[41] Complete dephytinzation via traditional fermentation to myo-inositol and inorganic phosphorus is unlikely, but lower myo-inositol phosphates that no longer inhibit zinc[36] or iron[37] absorption can be formed. By combining germination with lactic-acid fermentation, however, almost complete degradation of phytate in white sorghum and maize gruels has been achieved.[7] An additional advantage of fermented foods is their low pH and production of antimicrobial agents that inhibit the growth of pathogenic bacteria.[1,7,42]

Soaking cereals and legumes and discarding the soaking water can also reduce InsP6 through the passive diffusion of water-soluble sodium, potassium, and magnesium phytate[31,39,43] and through hydrolysis via activation of intrinsic plant phytases.[31,39] The extent of hydrolysis depends on the plant species and the conditions and duration of soaking: the activity of most intrinsic plant phytases is reportedly optimal between 40°C and 65°C and at a pH between 5.0 and 6.0.[31] Whether legumes or cereal grains are soaked intact or as finely ground flours or grits also affects the extent of the phytate loss.[44,45] For example, after soaking intact yellow mung beans (*Phaseolus radiatus*) at 30°C for 6 hours, no phytate (represented by the sum of InsP6 and InsP5) was lost, whereas 47% of phytate was lost after soaking mung beans milled as flour for the same duration.[44] Reductions of approximately 50% were also achieved after soaking unrefined white maize (*Zea mays*) milled either as a flour or pounded at approximately 25°C for 1 hour.[45] Here passive diffusion of water-soluble phytate was likely to be the predominant mechanism for phytate reduction because most of the phytate in maize is in the form of water-soluble sodium, potassium, or magnesium phytate.[43] After 1 hour of soaking, the proportion of InsP5 to InsP5 + InsP6 had increased to only 10% compared with 3% in the unsoaked maize flour control, with little further increase in the proportion of InsP5 to InsP5 + InsP6 after soaking for 24 hours.[45] These findings suggest that negligible hydrolysis of InsP6 via activation of intrinsic phytase had occurred, even though lower myo-inositol phosphate forms were not measured.[45] After soaking the flour or pounded maize, excess water is decanted, and the soaked flour or pounded maize is dried on a mat prior to cooking as a gruel or porridge in the household. Hence the growth of pathogenic bacteria under these conditions is unlikely.[42,45]

Heating the soaking water apparently activates intrinsic plant phytases during the early phase of cooking: positive correlations between phytase activity and phytate hydrolysis during the early phase of cooking have been reported.[39] Phytate reductions of 16%–24% have been reported, even when intact beans (*Phaseolus vulgaris)* were heated up to 65°C in the soaking water.[39,46] However, once the temperature is raised to the boiling point to complete the cooking process, the phytases will be inactivated and any pathogenic bacteria destroyed.[42] Some loss of minerals, water-soluble vitamins, and possibly polyphenols may also occur during soaking via leaching into the soaking medium.[45,47]

Hydrothermal treatment of whole grains is another processing method that can produce cereal products with a low phytate content. Historically, hydrothermal treatment of whole grains was practiced to facilitate the dehulling of cereals prior to milling. Originally, the treatment involved steeping in water, drying, and pounding the whole grains several times to remove the husk, a process that also led to some phytate degradation.[33] The process has been modified to maximize the degradation of phytate through activation of intrinsic phytases by optimizing the temperature for both the wet and dry steeps and adjusting the pH of the steeping water with lactic acid. Phytate reductions ranging from 84% to 99% in wheat, rye, and barley have been achieved after applying optimal conditions for their respective phytase activity.[33,34]

The phytate content of intact cereals and legumes may also be degraded by intrinsic phytase enzymes during storage[1,48] depending on the plant species, variety, and conditions and duration of storage. Legumes are more susceptible than cereals to phytate degradation, especially when stored at the temperatures and humidity often experienced in the tropics.[48] Decreases in InsP6 of 27% in beans (*Phaseolus vulgaris cv*) versus only 5%–10% in barley (*Hordeum vulgare*) have been reported after storage at 41°C and 75% humidity for 3 months compared with InsP6 levels after storage at room temperature.[48]

IMPACT OF HOUSEHOLD PHYTATE-REDUCING STRATEGIES ON MINERAL ABSORPTION

Very few isotope studies in humans have measured mineral absorption after applying the traditional household phytate-reducing strategies described above. An exception is traditional sour-dough fermentation used in baking rye bread, for which an increase in iron absorption associated with almost complete degradation of InsIP6 has been reported.[49]

Mineral absorption has also been measured in isotope studies on adults fed single meals[50,51] or whole-day diets[12] prepared with low-phytate maize with reductions in phytate levels similar to those achievable by traditional household processing, provided optimal conditions for phytase activity are used. For example, a low-phytate maize mutant (*lpa-1–1*) with approximately 60% phytate reduction improved absorption of zinc and calcium by 76%[12] and 43%,[50] respectively, whereas a low-phytate maize with a 35% phytate reduction achieved an approximately 50% increase in iron absorption.[51] However, in a longer-term study in which a low-phytate maize mutant (*Ipa-1–1*) was supplied to school-aged children (n = 20) in Guatemala for 10 weeks, zinc absorption was not increased in those who consumed the low-phytate maize mutant compared with those who consumed either the isohybrid wild-type maize (n = 20) or a local maize (n = 20) under the conditions of the study. The reason for these unexpected findings is uncertain.[52] Despite these reported improvements in mineral absorption, at least in adults, further exploration of this novel approach to enhance mineral absorption from plant-based diets has been hindered by the association of the low-phytate trait with reduced yields and by technical and cultural constraints, such as the need for long-term breeding projects specifically devoted to the low-phytate trait.[53]

Unlike with traditional household processing methods or the use of low-phytate maize mutants, complete degradation of phytate can sometimes be achieved by the addition of exogenous phytase enzymes, often from molds, specifically *Aspergillus niger*,[54] although intrinsic wheat phytase has also been used.[55] In these human isotopic feeding trials, phytase has been used to degrade phytate either during processing of the phytate-rich food or during stomach transit time when it has been incorporated as a functional ingredient in the test meal.[54] In the majority of cases, irrespective of the phytase method used to degrade phytate, marked increases in iron, zinc, and magnesium absorption in adults[54] and zinc and iron absorption in infants or young children[14,56,57] who consumed the dephytinized food compared with the high-phytate meals or diets have been reported. In the few studies with no substantial improvement in iron absorption, the low intrinsic phytate content of the test meal in combination with the presence of other iron absorption modifiers may have accounted for the absence of an effect.[56,58,59] For a detailed review of these studies, see Bohn et al[15] and Troesch et al.[54]

ANALYTICAL METHODS FOR PHYTIC ACID AND ITS DEPHOSPHORYLATED FORMS

Nonspecific or specific methods can be used to analyze InsP6 or its dephosphorylated forms. Nonspecific

Table 2 **Comparison of phytate content (mg/100 g fresh weight) and molar ratios of phytate-to-zinc of selected processed Ghanaian plant-based foods**

Common name	Botanical name and description of preparation	Phytate content by AOAC analysis, mg/100 g fresh weight[a]	Phytate content by HPLC analysis, mg/100 g fresh weight[b]	Phylate-to-zinc molar ratio byAOAC analysis[c]	Phylate-to-zinc molar ratio by HPLC analysis[d]
Banku	Partially fermented ground maize and grated cassava mixed as a dough and boiled	95	32	16	5
Ekoagbemi	Porridge prepared from dried corn kernels with skin and embryo removed, boiled for 1–2 h	23	12	23	12
Fu-fu	Cassava flour mixed with water and fermented 2–4 d, then blended to soft dough consistency as dumplings	96	20	24	5
Ga kenkey	Mixture of fermented maize meal and cooked maize dough prepared as dumplings and steamed in maize leaves	210	18	26	2
Gari	Dried cassava root, fermented 3–7 d, then ground into a flour, sieved, and fried	118	46	17	3
Sugar bread	White wheat flour bread: leavened	93	8	10	1

Data extracted from Ferguson et al (1993).[64]

Abbreviations: AOAC, Association of Analytical Chemists; HPCL, high-performance liquid chromatography.

[a]The AOAC method analyzes the sum of all myo-inositol phosphate forms by the anion-exchange method of Harland and Oberleas (1986).[61]

[b]The HPLC method **s**eparates and measures the InsP6 and InsP5 from lower inositol phosphate forms (Lehrfeld 1989).[63]

[c]Phytate-to-zinc molar ratios based on phytate analyzed by AOAC method (Harland and Oberleas 1986)[61] and zinc by atomic absorption spectrophotometry.

[d]Phytate-to-zinc molar ratios based on phytate represented by InsP6 and InsP5 analyed by HPLC (Lehrfeld 1989)[63] and zinc by atomic absorption spectrophotometry.

methods cannot distinguish InsP6 from its various dephosphorylated forms and instead measure the sum of all of the myo-inositol phosphates, irrespective of their form. In contrast, specific methods are capable of separating and quantifying individual inositol phosphate forms.

Many of the nonspecific methods are based on modifications of the ferric precipitation assay.[60,61] In this assay, after extraction, phytate is precipitated as ferric phytate, and following digestion, iron is removed from the ferric phytate precipitate, and the liberated InsP6 phosphorus is determined colorimetrically. The method assumes that all of the phosphate originates from InsP6 and none is derived from other phosphorylated compounds. In 1986, a modification was introduced to enhance the specificity of this assay and adopted as the official method by the Association of Analytical Chemists (AOAC) for determining phytate in foods.[61] The ferric-phytate precipitation step was omitted, and instead the phytate extract was purified by anion-exchange chromatography, yielding an elution fraction containing mainly InsP6 phosphorus.[61] The InsP6 content, termed more correctly "phytic acid equivalents," is calculated on the basis that 1 g of InsP6 phosphorus is equivalent to 3.55 g of InsP6.

Phytic acid is the most abundant form of myo-inositol phosphate found in mature, raw, unprocessed cereals, legumes, and oleaginous seeds.[1,62] Consequently, for raw, unprocessed plant-based foods, a nonspecific method based on modifications of the ferric precipitation assay is often used. During certain processing practices and storage, however, when InsP6 can be dephosphorylated to lower myo-inositol phosphate forms that no longer inhibit mineral absorption, nonspecific methods may yield values that are misleadingly high in relation to their potential to inhibit mineral bioavailability.[1,63] For example, as seen in Table 2,[61,63,64] the AOAC phytate values for Ghanaian fermented foods (Banku, Fu-fu, Ga kenkey, Gari), ekoagbemi prepared from dried stored maize, and leavened sugar bread[64] were all higher than the high-performance liquid chromatography (HPLC) values: the AOAC method measures not only InsP6 and its associated magnesium, calcium, and potassium phytate salts but also all of the other inositol phosphate forms (and nucleotides if present), which do not inhibit zinc and iron absorption.[65]

In contrast, HPLC is a specific method, capable of separating and quantifying the myo-inositol phosphate forms known to inhibit zinc and nonheme iron absorption from the lower myo-inositol phosphate forms that do not inhibit zinc or iron absorption.[37,65–67] Consequently, estimates of phytate intakes for a Ghanaian child consuming a serving of banku (260 g)

and 1 ball of kenkey (290 g) per day could range from 135 mg/day when based on HPLC represented by the sum of InsP5 + InsP6[63] to 856 mg/day when based on the AOAC 1986 method.[61]

Other specific methods besides HPLC capable of analyzing phytate and its dephosphorylated forms include thin-layer chromatography, gas-liquid chromatography, ion-exchange chromatography, and ^{31}P-nuclear magnetic resonance spectroscopy.[1,68–70] In the methods based on HPLC, after acidic extraction of phytate and other myo-inositol phosphates, anion exchange columns are used to purify and concentrate the extract, after which HPLC is used to separate and detect the individual inositol phosphate forms; more details are given in Schlemmer et al[1] and Lehrfeld.[63] Some HPLC methods are capable of quantifying InsP6–InsP4 or InsP6–InsP3 forms,[63,66,67] whereas others separate all of the inositol phosphate forms (ie, InsP6 to InsP1).[68] In the examples shown in Table 2, the HPLC data represent the sum of InsP6 and InsP5,[64] the two myo-inositol phosphate forms known to have a major inhibitory effect on zinc absorption, although during food processing (eg, fermentation, storage, and leavening), some of the lower myo-inositol phosphate forms (ie, InsP4 to InsP1) were likely to be formed. Of these, InsP4 and InsP3 are known to have a negative effect on iron[37] and possibly zinc[38] absorption.

Samples containing a high content of fat or oil may require defatting prior to phytate extraction to reduce potential interference by fat in the phytate extraction. Although HPLC is most frequently used, it may also be possible, using either thin-layer chromatography alone or in combination with a simplified version of the ferric precipitation method, to develop a low-cost, low-tech methodology that represents a specific method for phytate and inositol phosphate quantification. Such a low-cost/low-tech method might be more easily transferable to LICs than methods based on HPLC.

Marked differences exist in the literature for the "phytate" content of plant-based foods,[1,71] especially for those foods subjected to processing and storage. Such discrepancies are not surprising and can be attributed to differences in duration and conditions (eg, pH, temperature, humidity) of processing and storage, the botanical species and variety, stage of maturation, use of phosphate fertilizers, and the activity of intrinsic or extrinsic phytases. Methodological issues with the analysis of InsP6 and lower myo-inositol phosphate forms in foods are also a contributing factor. A new global food composition database compiled by the Food and Agriculture Organization of the United Nations (FAO)/International Network of Food Data Systems (INFOODS)/International Zinc Nutrition Consultative Group (IZiNCG) for InsP6 and lower myo-inositol phosphate forms (and iron, zinc, and calcium) of plant-based foods, together with details of the processing and analytical methods (designated by a specific tag name), is available, enabling users to select myo-inositol phosphate values based on the most appropriate processing and analytical method.[72]

IMPLICATIONS OF PHYTATE FOR ESTIMATING MINERAL BIOAVAILABILITY

The inhibiting effect of phytate on both zinc and iron absorption follows a dose-dependent response.[10,11,21] In early radioactive isotope studies on single cereal-based meals, zinc absorption was negatively correlated with phytate-to-zinc molar ratios and was approximately 50% less from diets with phytate-to-zinc molar ratios of 12–15 compared with diets with ratios <5.[11] This finding has been confirmed in a recent meta-analysis of 30 studies that examined the impact of phytate on zinc bioavailability: zinc absorption for test meals or diets with a phytate-to-zinc molar ratio of >15 was reported to be 45% of control values.[73] Molar ratios of phytate-to-zinc of individual foods or whole diets can be used to estimate the likely proportion of zinc absorbed.[8,74] Examples in Table 3[75–84] highlight the markedly lower phytate-to-zinc molar ratios for the diets of children based on fermented maize (in Ghana),[75] sago (in Papua New Guinea),[77] glutinous rice (in northeast Thailand),[78] and the consumption by omnivores (in Canada)[79,80] compared with the very high ratios for the unrefined, unfermented maize-based diets of the children in Malawi[75,85] and Kenya.[76]

Women from Malawi,[81] Ethiopia,[83] or India[84] consuming predominately maize[81,83] or rice and legumes[84] also have diets with elevated phytate-to-zinc molar ratios. Note the very low phytate-to-zinc molar ratios of the northeast Thai diets[78] arose because glutinous rice (*Oryza glutinosa*) is washed and soaked overnight before cooking, both practices that even further reduce the low phytate content of raw, milled rice by passive diffusion of water-soluble phytate into the water. For example, the analyzed InsP5 + InsP6 content of uncooked glutinous rice purchased from local vendors in northeast Thailand was 113 mg/100 g dry weight compared with 19.0 ± 16.5 mg/100 g dry weight for steamed polished glutinous rice.[78]

The negative effect of phytate on iron absorption is also dose dependent. However, inhibition of iron absorption occurs at lower concentrations of phytate (ie, 2–10 mg/meal phytate-phosphorous[21,22]) compared with the phytate concentrations that inhibit zinc absorption (50 mg/meal phytate-phosphorous).[10] Hence, for diets based on cereals and legumes, phytate-to-iron molar ratios less than at least 1:1, and preferably less

Table 3 **Intakes of dietary phytate, zinc, and iron and molar ratios of phytate-to-zinc and phytate-to-iron for young children and women**

Country (no. of participants)	Age, y[a] range or mean ± SD	Diet type	Phytate intake, mg/d[b]	Zinc intake, mg/d[b]	Phytate-to-zinc molar ratio[b]	Iron intake, mg/d[b]	Phytate-to-iron molar ratio[b]	References
Children								
Malawi (81)	4–7	Maize-based, unrefined[c]	1242 (874–1617)	6.0 (4.5–7.3)	21 (18–25)	9.6 (6.5–12.2)	12 (10–14)	Ferguson et al (1993)[75]
Kenya (138)	7.6 ± 0.3	Maize-based, unrefined[d]	2390 ± 480	7.2 ± 1.4	33 ± 3	14.5 ± 2.7	14	Murphy et al (1995)[76]
Egypt (63)	8.1 ± 0.6	Wheat-based, leavened[d]	1270 ± 280	8.0 ± 1.8	16 ± 2	8.0 ± 1.8	13	Murphy et al (1995)[76]
Ghana (76)	3–6	Maize-based, fermented[c]	393 ± 134	5.1 ± 1.1	8 ± 2	11.6 ± 2.4	0.2	Ferguson et al (1993)[75]
Papua New Guinea (67)	6–10	Sago-based[c]	570 ± 555	4.4 ± 1.2	13 ± 3	8.2 ± 3.3	6.0	Gibson et al (1991)[77]
Northeast Thailand (40)	6–13	Rice-based[e]	77 (66–109)	4.3 (3.7–6.1)	2 (1–2)	4.3 (3.2–6.5)	2.0	Krittaphol et al (2006)[78]
Canada (106)	4–6	Mixed[d]	~285[d]	6.9 ± 3.1	5	11.2 ± 4.4	2.0	Gibson et al (1991)[79] Smit Vanderkooy and Gibson (1987)[80]
Women								
Malawi: Pregnant: 24 wk (152)	23 ± 6	Maize-based[c]	1393 (942–2115)	9.0 (6.7–10.9)	17 (13–21)	14.8 (12.1–18.1)	8.0	Gibson and Huddle (1998)[81] Huddle et al (1999)[82]
Ethiopia: Pregnant: 36 wk (99)	28 ± 4.6	Maize + enset[c]	1011 (528–1575)	5.0 (3.3–7.2)	19 (13–24)	27.1 (20.7–33.2)	3 (2–5)	Abebe et al (2008)[83]
India (103)	18–30	Rice + legumes[d]	1264 (999–1795)	5.3 (3.8–7.0)	26 (22–28)	9.0 (6.0–12.9)	12	Herbst et al (2014)[84]

[a]Age is given as range or mean ± SD.
[b]Values for intakes and molar ratios are given as median (1st–3rd quartile) or mean ± SD.
[c]Diet type based on calculated intakes from analyzed values for local plant-based staples for both phytate (via high-performance liquid chromatography) and zinc.
[d]Diet type based on calculated intakes from literature values.
[e]Diet type based on analysis of duplicate diet composites for phytate (via high-performance liquid chromatography) and zinc.

than 0.4:1 are required before iron absorption is enhanced.[30] As noted in Table 3, the phytate-to-iron molar ratios reported for the predominantly plant-based diets[75–78] and even the mixed diets of the children in Canada[79,80] are all greater than 1:1, with the exception of the Ghanaian diets based on fermented maize.[75] For diets containing some enhancers of iron absorption, such as those for the children in Canada[79,80] and northeast Thailand,[78] molar ratios of phytate-to-iron less than 6:1 may suffice.[30]

Molar ratios for phytate-to-calcium and phytate-to-magnesium are rarely used to predict absorption, in part because of uncertainty over the critical molar ratios compromising absorption. In the past, millimolar ratios of phytate × calcium/zinc were also used to predict zinc absorption[86,87] because dietary calcium was said to influence the inhibitory effect of phytate on zinc bioavailability.[8] Their use has now been discontinued in the absence of any effect of calcium on zinc absorption, irrespective of whether intakes of dietary phytate are high or low.[88]

IMPLICATIONS OF PHYTATE FOR SETTING DIETARY REQUIREMENTS FOR ZINC

Phytate-to-zinc molar ratios of <5, 5–15, and >15 were used by the World Health Organization (WHO)[89] together with the amount and source of dietary protein to categorize zinc absorption of diets as potentially relatively high (50%), moderate (30%), or low (15%). These absorption estimates were used to set 3 levels of dietary zinc requirements based on data from published isotope studies on adults focused mainly on single meals, individual foods, and some whole-day diets.[90] More recent research has concluded that the phytate content of the diets has a more profound effect on zinc absorption than the protein content.[17,20] As a result, IZiNCG categorized diets exclusively on phytate-to-zinc molar ratios from data derived exclusively from whole-day diet studies of adults. Two diet types were defined, each with differing estimates for zinc absorption: mixed or refined vegetarian diets with ratios 4–18 (zinc absorption of 26% for males and 34% for females), and an unrefined vegetarian diet with a ratio >18 (zinc absorption 18% for males and 25% for females). Both WHO/FAO[89] and IZiNCG[17] used regression analysis to derive the adjustments necessary to generate the respective dietary zinc requirement (ie, the estimated average requirement) taking into account bioavailability for each diet type.

The negative effect of phytate on zinc absorption for adults is much higher than previously estimated.[20] As a result, the European Food Safety Authority (EFSA)[91] has generated new dietary zinc requirements for adults based on 4 levels of dietary phytate

Table 4 **Estimations of average requirements and population reference intakes for zinc according to phytate intake and body weight**

Phytate intake	Body weight, kg[a]	Average requirement, mg/d	Population reference intake, mg/d[b]
300 mg/d			
Women aged ≥18 y	58.5	6.2	7.5
Men aged ≥18 y	68.1	7.5	9.4
600 mg/d			
Women aged ≥18 y	58.5	7.6	9.3
Men aged ≥18 y	68.1	9.3	11.7
900 mg/d			
Women aged ≥18 y	58.5	8.9	11.0
Men aged ≥18 y	68.1	11.0	14.0
1200 mg/d			
Women aged ≥18 y	58.5	10.2	12.7
Men aged ≥18 y	68.1	12.7	16.3
Pregnancy			Additional 1.6
Lactation			Additional 2.9

Adapted from European Food Safety Authority, 2014, CC-BY-ND 4.0 with permission.[91]

[a]Body weight for women represents the median body weight of women aged 18–79 years based on measured body heights of 19 969 women in 13 European Union member states and assuming a body mass index of 22. Body weight for men represents the median body weight of men aged 18–79 years based on measured body heights of 16 500 men in 13 European Union member states and assuming a body mass index of 22.[91]

[b]Population reference intake represents the dietary zinc intake of individuals with a body weight at the 97.5th percentile of reference body weights (ie, 79.4 kg for men and 68.1 kg for women).

(Table 4).[91] A trivariate model of total absorbed zinc as a function of dietary zinc and dietary phytate based on saturation response modeling was used to evaluate the dietary zinc intake needed to meet physiological zinc requirements.[92]

The EFSA-estimated average requirement for zinc nearly doubles in adults when daily phytate intakes reach 1200 mg/day, a level surpassed by adults in some LICs who predominantly consume unfermented cereal and/or legume-based diets (Table 3). Therefore, more experimental data based on very high dietary phytate levels are needed to develop dietary zinc requirements for adults with phytate intakes >1200 mg/day.[90] Whether recommendations for dietary zinc based on phytate intakes can also be made for young children is uncertain and requires more investigation. Miller et al[29] failed to detect a negative effect of phytate on zinc absorption in their isotope studies of infants and young children. More details of the approaches used by WHO/FAO, IZiNCG, and EFSA to set dietary zinc requirements are available in Gibson et al.[90]

Because so many other dietary components besides phytate modify iron absorption,[30] phytate-to-iron molar ratios per se are not included in the WHO/FAO[89] model for predicting iron bioavailability. However, the WHO/FAO[89] algorithm does take into account the proportion of plant-based versus animal-source foods and ascorbic acid in diets, with absorption estimates ranging from 5% to 15% with increasing proportions of flesh foods and ascorbic acid in the diets.

Phytate is a component of several mathematical algorithms developed to predict nonheme iron absorption,[93] although almost all were derived from single-meal studies with the exception of the study by Armah et al.[94] For a detailed review of these algorithms, see Reddy.[95] A further limitation of all of the algorithms is that the values used generally represent the sum of all of the myo-inositol phosphate forms in foods rather than only those forms known to inhibit nonheme iron absorption (ie, InsP6 to InsP3). Using values for total inositol phosphates is likely to decrease the apparent negative impact of InsP6 in the models, thus compromising the ability of the models to accurately predict iron absorption.[94]

PROGRAM AND POLICY IMPLICATIONS OF HIGH-PHYTATE DIETS IN LOW-INCOME COUNTRIES

The WHO Guiding Principles on Infant and Young Child Feeding[96] recommend the introduction of complementary foods at 6 months of age together with continued breast feeding up to 2 years of age. In most LICs, complementary foods are prepared from a mixture of high-phytate unrefined cereal and legume flours, containing little or no animal-source foods. They thus have a low content of readily available iron and zinc and correspondingly high molar ratios of phytate-to-iron and phytate-to-zinc and hence do not meet the theoretical WHO-estimated needs for iron and zinc from complementary foods.[97] For a detailed review, see Gibson et al.[98] This is unfortunate because iron and zinc deficiency are major public health concerns in infants and young children in LICs. Iron deficiency contributes to anemia, which compromises, possibly irreversibly, psychomotor and mental development in young children,[99] whereas zinc deficiency impairs growth and increases the risk of morbidity and mortality.[100] Clearly, intervention strategies are needed to address these deficits in complementary foods in LICs.

Household dephytinization strategies have been adopted in 2 pilot studies conducted in rural Malawi. These strategies included the preparation of nsima (stiff maize porridge) and phalas (thin maize porridge) from soaked or fermented unrefined maize flour; soaking beans and discarding the soaking water prior to boiling; and enriching maize-based staples with animal-source foods, together with provitamin A– and vitamin C–rich foods.[85,101] Results indicated that, in addition to dephytinization strategies, increasing the consumption of

animal-source foods is necessary to overcome the deficits in iron and zinc in cereal-based diets of both infants[101] and young children[85] in rural settings in LICs. For a detailed review of other studies that have incorporated household dephytinization strategies, see Gibson and Anderson.[102]

Another way to overcome potential deficiencies of iron and zinc is to fortify cereal- and/or legume-based complementary foods. This can involve the use of micronutrient powders (MNPs; termed "sprinkles"),[103] fortified lipid-based spreads,[104] or the provision of processed fortified complementary foods distributed commercially or freely in national government programs.[105] Care must be taken to ensure that fortification levels are appropriate and do not exceed upper tolerable limits. An analysis of the mineral, InsP6, and InsP5 content of 27 processed complementary foods, 25 of which claimed to be fortified, revealed that almost none had the potential to meet the WHO-estimated needs for iron, zinc, or calcium for breastfed infants aged 9–11 months.[98,106] Nutritionists need to work with the food industry to enhance the quality of fortified, processed complementary foods.

Accumulating evidence indicates that the use of high doses of fortificant iron for young children may increase the risk of infection, notably diarrhea,[107] and has the potential to produce a more pathogenic gut microbiota profile and gut inflammation,[108,109] especially in settings with low standards of hygiene.[109] This concern has prompted investigation of possible strategies to reduce the dose of fortificant iron in MNPs while still delivering adequate levels. Three potential strategies have been investigated: 1) dephytinization through the addition of exogenous microbial phytases; 2) using a lower dose of iron (2.5 mg/d vs 12.5 mg/d) in the form of sodium iron ethylenediaminetetraacetate (NaFeEDTA), a form of iron with high bioavailability even in the presence of high-phytate foods; and 3) including, as an additional fortificant, ascorbic acid, a potent enhancer of nonheme iron absorption even in the presence of high levels of phytate and polyphenols.[110,111]

The revised MNP formulation (combined addition of phytase, ascorbic acid, and NaFeEDTA) was first tested in a stable isotope study of adult women; this study found a 5-fold increase in iron absorption from high-phytate maize porridge.[111] Next, an efficacy study in school children in South Africa[57] in which children received either a daily morning meal of high-phytate maize porridge fortified with the revised MNP formulation (2.5 mg/d of iron as NaFeEDTA and 2.5 mg zinc as zinc oxide, plus phytase) or a placebo on each school day for 23 weeks was conducted. At endline, the prevalences of both iron deficiency and zinc deficiency were reduced ($P < 0.05$), and weight-for-age Z scores increased in the treatment group compared with the control group ($P < 0.05$). Nevertheless, even though the lower doses of fortificant iron (and zinc) were shown to be efficacious in this setting, thus alleviating safety concerns over the use of high doses of fortificant iron, barriers still remain to the use of microbial phytase in the revised MNP formulation. The phytase enzyme was derived from genetically modified *Aspergillus niger*, selected because it remains stable even at high temperatures and at the acid pH of the stomach and has been classified by the US Food and Drug Administration as "generally recognized as safe–self-affirmed." However, to date, many LICs have restrictions on the use of genetically modified organisms.

Despite recognition that zinc deficiency is likely to be widespread in LICs, at least partly due to poor bioavailability of dietary zinc, the prevalence of zinc deficiency in many LICs remains unknown. In response to this concern, Wessells and Brown[112] have generated regional and national estimates for risk of zinc deficiency based on the prevalence of inadequate intakes of dietary zinc. These estimates were based on the predicted absorbable zinc content of the national daily food supply calculated as a function of dietary zinc and phytate from 2011 FAO food balance sheet data. When the prevalence of inadequate zinc intakes is greater than 25%, the population is considered to be at high risk of zinc deficiency.[113] Regions identified at highest risk are in sub-Saharan Africa and South and Southeast Asia. With access to accurate analytical data on the InsP6 and InsP5 content of both unprocessed and processed plant-based foods combined with nationally representative food consumption survey data, national estimates for risk of zinc deficiency among infants and young children and women of reproductive age can be generated. These estimates can be used to implement targeted policies to alleviate risk of zinc deficiency among these vulnerable population groups.

CONCLUSION

In LICs, diets are often plant based and, as a result, have a high content of phytate, a potent inhibitor of iron and zinc absorption. Traditional food-processing methods and storage have the potential to reduce the phytate content of cereal and legume staples through loss of water-soluble phytate by diffusion and through phytase-induced hydrolysis to lower inositol phosphate forms, some of which no longer inhibit iron and zinc absorption. Hence, for stored and processed foods, analytical methods such as HPLC or, possibly, lower-tech alternatives such as thin-layer chromatography that are capable of separating and quantifying the myo-inositol phosphate forms known to inhibit iron and zinc absorption

should be used. Dietary phytate-to-zinc and phytate-to-iron molar ratios can be used to estimate the bioavailability of iron and zinc in individual foods or whole diets and to design food-based strategies to enhance bioavailability of zinc and iron. With the advent of new dietary zinc requirements based on 4 levels of phytate intake for adults, there is an urgent need to generate accurate data on phytate intakes in LICs. Hence, appropriate food composition values that take into account food-processing and analytical methods must be used. Increasingly, foods are being fortified with micronutrients to overcome deficits in iron and zinc, especially in complementary foods for infant and young child feeding. Research is focused on strategies to lower the iron fortificant dose required to reduce the risk of iron deficiency while simultaneously alleviating safety concerns. Finally, enhanced estimates of risk of zinc deficiency based on the prevalence of inadequate zinc intakes among vulnerable groups will facilitate the implementation of targeted policies for alleviating risk of zinc deficiency in LICs.

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