

## Improving the bioavailability of nutrients in plant foods at the household level

Rosalind S. Gibson\*, Leah Perlas<sup>†</sup> and Christine Hotz<sup>‡</sup>  
*Department of Human Nutrition, University of Otago, Dunedin, New Zealand*

Plant foods are the major staples of diets in developing countries, in which the consumption of animal-source foods is often low because of economic and/or religious concerns. However, such plant-based diets are often associated with micronutrient deficits, exacerbated in part by poor micronutrient bioavailability. Diet-related factors in plant foods that affect bioavailability include: the chemical form of the nutrient in food and/or nature of the food matrix; interactions between nutrients and other organic components (e.g. phytate, polyphenols, dietary fibre, oxalic acid, protein, fat, ascorbic acid); pretreatment of food as a result of processing and/or preparation practices. Consequently, household strategies that reduce the content or counteract the inhibiting effects of these factors on micronutrient bioavailability are urgently needed in developing-country settings. Examples of such strategies include: germination, microbial fermentation or soaking to reduce the phytate and polyphenol content of unrefined cereal porridges used for young child feeding; addition of ascorbic acid-containing fruits to enhance non-haem-Fe absorption; heating to destroy heat-labile anti-nutritional factors (e.g. goitrogens, thiaminases) or disrupt carotenoid–protein complexes. Such strategies have been employed in both experimental isotope-absorption and community-based studies. Increases in Fe, Zn and Ca absorption have been reported in adults fed dephytinized cereals compared with cereals containing their native phytate. In community-based studies in rural Malawi improvements in dietary quality and arm-muscle area and reductions in the incidence of anaemia and common infections in young children have been observed.

### **Bioavailability: Plant foods: Household: Micronutrients: Phytate**

In developing countries plant foods are the major staples of the diet and consumption of animal-source foods is often low because of economic and/or religious concerns. Such plant-based diets are, however, often associated with deficits in Ca, Fe, Zn and some vitamins. A major factor contributing to these deficits, particularly for diets based on unrefined cereals and legumes, is that bioavailability, which can be defined as the proportion of an ingested trace element in food that is absorbed and utilized for normal metabolic and physiological functions or storage (Jackson, 1997), is poor. Bioavailability is influenced by both dietary and host-related factors (Fairweather-Tait & Hurrell, 1996). The present review addresses the dietary factors and summarizes food preparation and processing practices that can be used in the household to enhance nutrient

bioavailability. Examples of efficacy studies employing these strategies in developing countries are also given.

### **Diet-related factors in plant foods that affect bioavailability**

Several dietary factors may affect the nutrient bioavailability of plant foods when they are consumed, including: (1) the chemical form of the nutrient in the food and the nature of the food matrix; (2) interactions occurring between nutrients and other organic components within the plant food; (3) pretreatment of the food during processing and/or preparation.

\*Corresponding author: Professor R. S. Gibson, fax +64 3 479 7958, email Rosalind.Gibson@stonebow.otago.ac.nz

<sup>†</sup>Present address: Food and Nutrition Research Institute, Department of Science and Technology, Bicutan, Taguig, Metro Manila, Philippines.

<sup>‡</sup>Present address: HarvestPlus, c/o International Food Policy Research Institute, 2003 K Street, NW, Washington DC 20006-1002, USA.

**Table 1.** Effects of non-competitive interactions involving organic substances on nutrient bioavailability in plant foods: inhibiting factors

Dietary component	Food sources	Main technical influences	Nutritional consequences
Phytate ( <i>myo</i> -inositol hexaphosphate) plus magnesium, calcium or potassium phytate	Unrefined cereals, legumes, nuts, oil seeds	Binds certain cations to form insoluble complexes in gut	Zn, Fe, Ca and probably Mg are poorly absorbed (Heaney <i>et al.</i> 1991; Sandberg <i>et al.</i> 1999)
Soyabean protein	Some varieties of soyabeans, unfermented tofu, textured vegetable protein	Effect not explicable on basis of phytate content but instead depends on variety and processing method	Inhibits Fe and Zn absorption in some varieties. Some contain Fe as phytoferrin, which may be highly bioavailable (Murray-Kolb <i>et al.</i> 2003)
Polyphenols	Certain cereals (red sorghum), legumes (red kidney beans, black beans, black grams), spinach, betel leaves, oregano Beverages: tea, coffee, cocoa, red wine	Form insoluble complexes with Fe Some polyphenols inactivate thiamin Bind certain salivary and digestive enzymes Enhance excretion of endogenous protein	Inhibit non-haem-Fe absorption Reduce thiamin absorption Reduce digestibility of starch, protein and lipids Interfere with protein digestibility (Bravo, 1998)
Oxalic acid	Amaranth, spinach, rhubarb, yam, taro, sweet potato, sorrel, sesame seeds, black tea	Oxalates form insoluble complexes with Ca and possibly Fe	Reduce absorption of Ca and possibly Fe; increase urinary Ca (Savage, 2002)
Dietary fibre	Unrefined cereals, legumes, nuts, oil seeds, fruits and vegetables	Lignin and pectin bind bile acids Pectins, psyllium and gums retain water and form viscous solutions in gastrointestinal tract Dietary fibres are fermented in large intestine by microflora	Reduces absorption of fats, fat-soluble vitamins and carotenoids; effects on folate bioavailability inconsistent Slows gastric emptying and digestion and absorption of nutrients (Gallagher & Schneeman, 2001) SCFA produced that enhance Ca solubility (Demigne <i>et al.</i> 1995)

Sorghum, *Sorghum bicolor* (L.) Moench; red kidney beans, *Phaseolus vulgaris*; black beans, *Glycine max*; black gram, *Phaseolus mungo*; spinach, *Spinacia oleracea*; betel, *Piper betel*; oregano, *Origanum vulgare*; amaranth, *Amaranthus edulis*; rhubarb, *Rheum rhaponticum*; yam, *Dioscorea* spp.; taro, *Colocasia esculenta* var. *antiquorum*; sweet potato, *Ipomoea batatas*; sesame, *Sesamum orientale*.

In general, diet-related factors have a greater influence on the bioavailability of the micronutrients in plant foods, particularly Ca, Fe and Zn, than on the macronutrients. The absorption of Ca, Fe and Zn is particularly affected. The net effect on the nutrient bioavailability depends on the balance between factors that either inhibit or enhance nutrient absorption and/or utilization in the whole diet (Sandström, 2001). Increasingly, the influence of both synthetic micronutrient fortificants and intrinsic micronutrients on micronutrient bioavailability must be considered.

#### *Chemical form of the nutrient and nature of the dietary matrix*

The absorption and/or utilization of the trace elements Fe, Se and Zn, and of the vitamins niacin, provitamin A carotenoids and folate are most affected by their chemical form. Of these micronutrients, the bioavailability of the two forms of Fe in food (haem- and non-haem-Fe; Hallberg, 1981), certain isomeric forms of carotenoids (Yeum & Russell, 2002) and folate polyglutamates relative to monoglutamates (McNulty & Pentieva, 2004) have been reviewed in detail elsewhere.

The food matrix probably has the greatest effect on the absorption of provitamin A carotenoids and folate, both of which can be entrapped in the insoluble plant matrix, thus reducing their bioavailability. For example,  $\beta$ -carotene in

raw carrots or lycopene in fresh tomato juice are poorly absorbed compared with pure  $\beta$ -carotene dissolved in oil (Yeum & Russell, 2002), whereas the bioavailability of folate from chopped spinach (*Spinacia oleracea*) is higher than that from whole spinach leaves (Castenmiller *et al.* 2000).

#### *Interactions between nutrients themselves and with other organic components in the plant food*

Direct competitive interactions between two (or more) inorganic nutrients with similar physico-chemical properties that share the same absorptive pathways are unlikely in plant foods because the intrinsic micronutrient levels are low. Even when plant staples serve as vehicles for fortification the risk of such interactions is small because of the presence of dietary ligands in food (Sandström *et al.* 1985).

In contrast, there are several organic components in plant foods that may form insoluble or soluble complexes with certain micronutrients in the acid pH of the stomach and gastrointestinal tract, thus inhibiting or facilitating their absorption. Re-absorption of endogenously-excreted Ca, Zn and Cu may also be affected (Sandström, 2001; Manary *et al.* 2002a). Examples of these non-competitive interactions are summarized in Tables 1 and 2; both inhibiting and enhancing factors are listed.

**Table 2.** Effects of non-competitive interactions involving organic substances on nutrient bioavailability in plant foods: enhancing factors

Dietary component	Food sources	Main technical influences	Nutritional consequences
Organic acids (citric, lactic, acetic, butyric, propionic and formic acids)	Fermented milk products (e.g. yoghurts), vegetables, sauerkraut, soya sauces, fermented cereals (e.g. Tobwa)	May form soluble ligands with some trace minerals in the gastrointestinal tract	Enhance absorption of Zn and possibly Fe (Sandström, 1997; Teucher <i>et al.</i> 2004)
Ascorbic acid	Citrus fruits and juices Other fruits: guavas, mango, papayas, kiwi, strawberries Vegetables: tomato, asparagus, Brussels sprouts, spinach etc.	Reduces Fe <sup>3+</sup> to more soluble Fe <sup>2+</sup> ; forms Fe–ascorbate chelate  May increase stability of folate during food processing and digestion	Enhances non-haem-Fe absorption (Teucher <i>et al.</i> 2004) Counteracts inhibitory effect of phytate May enhance folate bioavailability (McNulty & Pentieva, 2004) May enhance or inhibit Se absorption, depending on the chemical form (Mutanen & Mykkanen, 1985; Levander, 1987). Ascorbic acid may also enhance Cr absorption (Offenbacher, 1994)
Protein		Amount and type (e.g. animal protein) form soluble ligands with Zn, Fe and Cu	Enhance absorption of Zn, Fe and Cu (Bjorn-Rasmussen & Hallberg, 1979; Turlund <i>et al.</i> 1983; Lönnerdal, 2000) Increase urinary Ca excretion (Heaney, 2000)
Fat	Oil seeds, nuts	Products of fat digestion + bile salts solubilize fat-soluble vitamins and carotenoids in intestinal milieu	Enhance absorption of fat-soluble vitamins and provitamin A carotenoids (Yeum & Russell, 2002)

Guava, *Psidium guajava* L.; mango, *Mangifera indica* L.; papaya, *Carica papaya*; kiwi, *Actinidia deliciosa*; strawberry, *Fragaria X ananassa*; asparagus, *Asparagus officinalis*; spinach, *Spinacia oleracea*.

#### *Pretreatment of food in the household as a result of processing and/or preparation practices*

The adverse effects of some of the organic components in plant foods on nutrient bioavailability can be reduced by household food processing and preparation practices; these practices are summarized in Table 3 and will be discussed.

**Thermal processing.** This treatment generally enhances the digestibility of proteins and carbohydrates, although if Maillard browning occurs in baked foods protein quality and digestibility may be reduced (Table 3). Thermal processing may also improve the bioavailability of certain vitamins and I, in some cases because of the destruction of heat-labile anti-nutritional factors (Erdman & Pneros-Schneier, 1994). For example, thiaminases in Brussels sprouts and red cabbage, which catalyse the cleavage of thiamin, are destroyed by cooking (Hilker & Somogyi, 1982). Cooking also destroys the goitrogens present in cabbage, Brussels sprouts, turnips, sweet potatoes (*Ipomoea batatas*), millet (*hatherum hymenoides*), cassava (*Manihot esculenta* Crantz) and beans. Such goitrogens block the absorption or utilization of I and thus its uptake into the thyroid gland (Gaitan, 1990).

Thermal processing can also enhance the bioavailability of vitamins such as thiamin, vitamin B<sub>6</sub>, niacin and carotenoids by releasing them from entrapment in the plant matrix. For example, greater increases in total serum

β-carotene and serum lycopene have been reported after eating cooked carrots and spinach (Rock *et al.* 1998) and cooked tomatoes (van het Hof *et al.* 2000) compared with levels when they are consumed raw. This effect is attributed to softening or disruption of plant cell walls and the disruption of carotenoid–protein complexes so that the carotenoids are more available in the intestinal lumen for absorption (Yeum & Russell, 2002).

Reports on the effects of thermal processing on phytate degradation are inconsistent and depend on the plant species, temperature and/or pH. Hurrell *et al.* (2002) have reported that home thermal processing does not degrade phytate sufficiently to improve Fe absorption from home-prepared pancakes or chapattis. Other investigators (Kataria *et al.* 1989; Marfo *et al.* 1990; Khan *et al.* 1991) have suggested that conventional heat treatments such as boiling may induce moderate losses (i.e. 5–15%) of phytic acid in tubers (Marfo *et al.* 1990) and some legumes (Kataria *et al.* 1989) and cereals (Khan *et al.* 1991). Much higher losses have been reported after boiling white rice (i.e. 70%; Perlas & Gibson, 2002), attributed mainly to leaching of water-soluble sodium, potassium or magnesium phytate into the discarded cooking water.

**Germination.** This process, also termed malting, leads to an increase in phytase activity in certain cereals (e.g. maize, millet and sorghum (*Sorghum bicolor* (L.) Moench)), in most legumes and in oil seeds through *de novo* synthesis and/or activation of intrinsic phytase.

**Table 3.** Influence of household food processing and preparation methods on bioavailability of nutrients in plant foods

Processing method	Main technical influences	Nutritional consequences
Thermal processing	Releases some vitamins from poorly-digested complexes Inactivates heat-labile anti-nutritional factors (e.g. protease inhibitors, $\alpha$ -amylase inhibitors, lectins, thiaminases, goitrogens) May degrade phytate, depending on temperature Gelatinizes starch	Enhances bioavailability of vitamin B <sub>6</sub> , niacin, folate and certain carotenoids Enhances digestibility of proteins and starch Enhances bioavailability of thiamine and I  May enhance Zn, Fe and Ca bioavailability Enhances digestibility
Baking	Induces Maillard browning in foods containing reducing sugars	Destroys basic essential amino acids lysine, arginine and methionine Reduces protein quality and protein digestibility
Boiling	Reduces oxalate content	Enhances Ca absorption
Germination and malting	Increases phytase activity via <i>de novo</i> synthesis or activation of endogenous phytase Reduces polyphenol content of some legumes (e.g. <i>Vicia faba</i> ) Increases $\alpha$ -amylase content of cereals (e.g. sorghum and millet)	Induces hydrolysis of phytate and hence increases Zn, Fe, Ca, and Mg absorption Enhances non-haem-Fe absorption  Facilitates starch digestion; may increase non-haem-Fe absorption through a change in consistency
Village-based milling or home pounding	Reduces phytate content of cereals with phytate localized in outer aleurone layer (rice, wheat, sorghum) or in germ (maize)	Enhances bioavailability of Zn, Fe, and Ca, although mineral content simultaneously reduced
Microbial fermentation	Induces hydrolysis of phytate by microbial phytase Increases content of organic acids  Microbial enzymes may destroy protein inhibitors that interfere with N digestibility	Enhances bioavailability of Zn, Fe and Ca May form soluble ligands with non-haem-Fe and Zn, and enhance bioavailability May improve protein quality in maize, legumes, groundnuts and pumpkin and millet seeds

Sorghum, *Sorghum bicolor* (L.) Moench; millet, *Achnatherum hymenoides*; groundnut, *Apios americana* Medic.; pumpkin, *Cucurbita Pepo*.

Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, wheat, triticale (*X Triticosecale* Wittmack), buckwheat (*Fagopyrum esculentum*) or barley (Egli *et al.* 2002). Phytases (*myo*-inositol hexakisphosphate 3-phosphohydrolase) hydrolyse phytic acid (*myo*-inositol hexaphosphate) as well as the salts, magnesium, calcium or potassium phytate, to yield inorganic orthophosphate and *myo*-inositol via intermediate *myo*-inositol phosphates (pentaphosphates to monophosphates). The rate of phytate hydrolysis varies with the species and variety, as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57°C), solubility of phytate and the presence of certain inhibitors (Cheryan, 1980; Egli *et al.* 2003). Egli *et al.* (2002) have observed that during germination rice, millet and mungbean (*Vigna radiata* L.) have the largest reductions in phytate content, ranging from 50% (for mungbeans) to 64%.

Such reductions in the levels of higher inositol phosphates can have a major impact on mineral bioavailability because they form complexes with divalent and trivalent cations (particularly Zn, Fe, Ca and Mg) at the physiological pH conditions of the small intestine, making them unavailable for absorption (Oberleas & Harland, 1981; Hurrell, 2003; Egli *et al.* 2004; Hurrell *et al.* 2004); the higher inositol phosphates have no effect on Cu absorption (Egli *et al.* 2004). The hexa- and pentaphosphates may also complex endogenously-secreted minerals such as Zn

(Sandström, 1997; Manary *et al.* 2000) and Ca (Morris & Ellis, 1985), making them unavailable for re-absorption into the body. In contrast, *myo*-inositol phosphates with less than five phosphate groups (i.e. monophosphates to tetraphosphates) do not have a negative effect on Zn absorption (Lönnerdal *et al.* 1989) and those with less than three phosphate groups do not inhibit non-haem-Fe absorption (Sandberg *et al.* 1999). There appears to be no adaptation to the inhibitory effect of a high-phytate diet on absorption of Fe (Brune *et al.* 1989) or exogenous Zn, although endogenous excretion of faecal Zn may be decreased in healthy subjects (Sandström *et al.* 1993).

Certain tannins and other polyphenols in legumes (e.g. *Vicia faba*) and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho *et al.* 1992). Naturally-occurring polyphenol oxidase extracted from banana (*Musa X paradisiaca* L.) or avocado (*Persea americana* Mill.) and subsequently reduced by dialysis has also been used to reduce the polyphenol content of high-tannin sorghum (Matuschek & Svanberg, 2004).

$\alpha$ -Amylase activity is also increased during germination of cereals, particularly sorghum and millet. This enzyme hydrolyses amylose and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges (Gibson & Ferguson, 1998). A threefold increase in Fe absorption has been reported in amylase-treated



roller-dried rice cereal compared with the untreated roller-dried cereal, which is associated with the viscosity changes induced by  $\alpha$ -amylase (Hurrell *et al.* 2002).

**Milling or household pounding.** In developing countries this process is used to remove the bran and/or germ from cereals such as wheat, sorghum, rice and maize. These processes also reduce the phytate content if the phytate is localized in the outer aleurone layer (e.g. rice, sorghum and wheat) or in the germ (i.e. maize; O'Dell *et al.* 1972). Milling can thus enhance mineral bioavailability, although the content of minerals and some vitamins of the milled cereals are simultaneously reduced. As a result, in some countries milled cereal flours are enriched to compensate for the micronutrients lost.

**Microbial fermentation.** Higher inositol phosphates are hydrolysed to lower inositol phosphates through the action of microbial phytase enzymes (Sandberg, 1991). These micro-organisms may occur naturally on the surface of cereals and legumes or can be introduced via inoculation with a starter culture. The extent of the reduction in higher inositol phosphate levels depends on the type of fermentation; sometimes  $\geq 90\%$  phytate can be removed by fermentation of maize, soyabeans, sorghum, cassava, cocoyam (*Colocasia esculenta*), cowpeas (*Vigna unguiculata*) and lima beans (*Phaseolus limensis*; Marfo *et al.* 1990; Sandberg, 1991; Svanberg *et al.* 1993). Fermentation of bread dough with yeast also induces phytate hydrolysis, although if Ca is added as a fortificant phytase activity in yeast is inhibited (Hallberg *et al.* 1991).

Organic acids are also produced during fermentation and can potentially enhance Fe and Zn absorption via the formation of soluble ligands (Charlton, 1983; Hazell & Johnson, 1987; Walter *et al.* 1998; Porres *et al.* 2001). They may also complex some of the minerals bound to phytate molecules, rendering them more susceptible to hydrolysis via phytase enzymes (Maenz *et al.* 1999), while simultaneously generating a pH that optimizes the activity of intrinsic phytase from cereal or legume flours (Porres *et al.* 2001). In contrast, organic acids may have an inhibitory effect on the activity of the intestinal brush-border enzyme glutamate carboxypeptidase II, attributed to a lowering of the pH (McNulty & Pentieva, 2004).

Improvements in protein quality have also been documented after fermenting blended mixtures of plant-based complementary foods based on maize and legumes (Nnam, 1999), groundnuts (*Apios americana* Medic), pumpkin (*Cucurbita Pepo*) and millet seeds (Ezeji & Ojmelukwe, 1993) and cereal and soyabean blends (Sanni *et al.* 1999). Such improvements may be associated with the destruction by microbial enzymes of protein inhibitors that interfere with N digestibility (Nnam, 1999), or from the ability of starter cultures to synthesize certain amino acids (Odunfa, 1985).

**Soaking.** The soaking of cereal and most legume flours in water results in the passive diffusion of water-soluble sodium, potassium or magnesium phytate, which can be removed by decanting the water (De Boland *et al.* 1975; Chang *et al.* 1977; Perlas & Gibson, 2002). Nevertheless, the extent of the removal depends on the species, pH and length and conditions of soaking. Reductions in the penta- and hexaphosphates of 47, 57 and 98% respectively have

been reported for mungbean, maize and rice flours after soaking (Hotz & Gibson, 2001; Perlas & Gibson, 2002; Temple *et al.* 2002); however, no reductions are achieved after soaking whole mungbeans and maize kernels for 6 h (Perlas & Gibson, 2002; Temple *et al.* 2002). Reductions in the content of other anti-nutrients such as glycosides, alkaloids, oligosaccharides, saponins, polyphenols and oxalates may also occur (Chang *et al.* 1977).

### Application of household processing and preparation strategies to enhance nutrient bioavailability of plant foods in developing countries

There is an urgent need to improve the nutritional quality of plant-based foods in developing countries, especially those used for feeding infants and young children. In the past the emphasis has been on enhancing their protein quality by blending cereals with legumes (usually in ratios of 70:30 (w/w) to provide the optimal mixture of essential amino acids), and problems associated with mineral bioavailability have often been ignored. This approach is unfortunate because many of these cereal-legume blends have a very high phytate content and high phytate:Zn and phytate:Fe molar ratios.

The inhibitory effect of phytate on Zn absorption follows a dose-dependent response (Navert *et al.* 1985) and the molar ratio for phytate:Zn in the diet is used to estimate the proportion of absorbable Zn (Oberleas & Harland, 1981). For Fe, phytic acid begins to lose its inhibitory effect on Fe absorption when ratios are  $< 1.0:1.0$  and it still inhibits Fe absorption at ratios as low as  $0.2:1.0$  (Hallberg *et al.* 1989; Hurrell *et al.* 1992).

Both *in vitro* and *in vivo* methods have been used to estimate the bioavailability of Fe, Zn and Ca in plant foods. Some *in vitro* methods are based on a two-stage simulated digestive process of the food or test meal, followed by determination of the dialysable Fe, Zn or Ca released. In general, the magnitude of the responses measured using these methods are not the same as those observed in human subjects, but some of these methods have been used to rank foods with respect to the effect of processing and preparation practices on mineral bioavailability (Latunde-Dada *et al.* 1998). For example, increases in dialysable Fe, Zn and Ca have been reported after processing porridges prepared from legumes such as chickpea (*Cicer arietinum*) and black gram (*Phaseolus mungo*) flours and/or cereal flours such as maize, sorghum and rice by fermentation with a starter culture (Svanberg *et al.* 1993; Jood & Kapoor, 1997; Sharma & Khetarpaul, 1998) and/or by soaking and germination (Svanberg *et al.* 1993; Mbithi-Mwikya *et al.* 2002).

More recently, cultured human intestinal cells (i.e. a Caco-2 cell *in vitro* model) have been developed for studying the characteristics of Fe, and in some cases Zn and Ca, transport by the intestinal absorptive epithelium (Han *et al.* 1994; Wortley *et al.* 2005). However, currently, there is no standardized Caco-2 cell method and the magnitude of the effects observed appears to depend on the procedures used, making inter-laboratory comparisons difficult. Studies have applied this technique to screen and

rank selected staple-food genotypes for bioavailable Fe (Van Campen & Glahn, 1999), but further development of the Caco-2 cell model is needed before it can be used to determine the bioavailability of Zn and provitamin A carotenoids in plant foods.

To date, *in vivo* isotope studies to measure the bioavailability of Fe or Zn in plant foods modified using household strategies to enhance Fe or Zn absorption are limited. Studies on adults have demonstrated increases in Fe and Zn absorption when they are fed porridges used for infant feeding that have been prepared from a variety of dephytinized cereals as compared with those containing their native phytate. In a study of Fe absorption (Hurrell *et al.* 2003) phytic acid was degraded by the addition of exogenous commercial phytase enzyme, whereas in a Zn-absorption study (Egli *et al.* 2004) dephytinization of a wheat–soyabean blend was achieved by the use of phytase naturally occurring in wholegrain cereals (Egli *et al.* 2003). In a hospital-based study in Malawi (Manary *et al.* 2000) the reduction of phytate in a maize–soyabean porridge by using a commercial phytase enzyme has been shown to increase fractional and total Zn absorption and reduce endogenous Zn losses in children recovering from tuberculosis but has no effect on Zn absorption in apparently-well children.

Only a few community-based efficacy trials have assessed the impact of food-based strategies in the household designed to enhance nutrient bioavailability. Early studies focused on improving the bioavailability of non-haem-Fe have been reviewed by Ruel (2001). More recent studies have measured various outcomes, including absorption *in vivo* using stable isotopes of Fe (Diaz *et al.* 2003), nutrient adequacy (Gibson *et al.* 2003; Hotz & Gibson, 2005) and biochemical and/or functional health outcomes (Dewey *et al.* 1997; Manary *et al.* 2002b; Yeudall *et al.* 2002; Garcia *et al.* 2003; Mamiro *et al.* 2004).

In a recent study in rural Mexico no improvement in biochemical Fe status was observed among Fe-deficient women receiving 25 mg ascorbic acid from fresh lime juice twice daily on 6 d/week for 8 months compared with those receiving a placebo (Garcia *et al.* 2003), despite a twofold increase in Fe absorption, based on earlier stable-isotope results (Diaz *et al.* 2003). Similarly, after withholding coffee for 5 months no positive effect on Fe status was observed among Fe-deficient Guatemalan toddlers, except among those taking Fe supplements (Dewey *et al.* 1997), attributed to the relatively small amount of coffee ingested. Furthermore, in a large community-based double-blind randomized controlled trial in Tanzania (Mamiro *et al.* 2004), in which a processed complementary food (based on soaked and germinated finger millet (*Eleusine coracana*) and kidney beans (*Phaseolus vulgaris*), with roasted peanuts (*Arachis hypogea*) and mango (*Mangifera indica* L.) puree) and an identical unprocessed blend were fed to 6-month-old infants (*n* 309) for 6 months, no significant differences were found between the two groups at the end of the study in either Fe status, as measured by Hb and zinc protoporphyrin, or growth, perhaps in part because there was only a 34% reduction in the phytate content of the processed complementary food.

These results emphasize that an integrated approach that combines a variety of the strategies discussed earlier, including the addition of even a small amount of animal-source foods, is probably the best strategy to improve the nutrient bioavailability in diets based on plant foods. Two such community-based efficacy trials have been undertaken among weanlings and young children in rural Malawi. Both trials employed a quasi-experimental design with non-equivalent control groups and used a participatory approach to implement a combination of food-based strategies in the household to enhance their awareness, feasibility and acceptability to caregivers in the local community. Details of the strategies and their implementation have been published (Gibson *et al.* 1998, 2003; Yeudall *et al.* 2002, 2005; Hotz & Gibson, 2005); their efficacy was evaluated by determining knowledge, trial and adoption of the new practices and comparing dietary quality and the adequacy of the energy and nutrient intakes of the intervention and control groups post-intervention (Gibson *et al.* 2003; Hotz & Gibson, 2005) and, for the children only, changes in growth and body composition, morbidity and Hb and hair Zn concentrations (Yeudall *et al.* 2002).

Results of the Malawian studies suggest that a combination of household food-based strategies, comparable with those outlined earlier, can be designed to be feasible and acceptable to caregivers of weanlings and children in subsistence farming settings, although on-going nutrition education and social marketing efforts are required to enhance their adoption and to empower the community to sustain them. Nevertheless, even when such a combination of strategies is used, they are probably not sufficient to overcome the deficits in Ca, Fe and Zn, and possibly other micronutrients that exist in complementary diets in low-resource settings. In such cases additional strategies to enhance the micronutrient adequacy of these complementary diets are urgently required. Possible strategies include fortifying cereal-based dephytinized complementary foods with a fortificant containing balanced and physiological levels of multi-micronutrients. In the long term, biofortification of staple cereals involving strategies to enhance both micronutrient density and bioavailability may become a feasible option for improving the micronutrient status of the entire household in poor-resource settings.

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