Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops

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International research efforts, including those funded by HarvestPlus, a Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), are focusing on conventional plant breeding to biofortify staple crops such as maize, rice, cassava, beans, wheat, sweet potatoes, and pearl millet to increase the concentrations of micronutrients that are commonly deficient in specific population groups of developing countries. The bioavailability of micronutrients in unfortified staple crops in developing regions is typically low, which raises questions about the efficacy of these crops to improve population micronutrient status. This review of recent studies of biofortified crops aims to assess the micronutrient bioavailability of biofortified staple crops in order to derive lessons that may help direct plant breeding and to infer the potential efficacy of food-based nutrition interventions. Although reducing the amounts of antinutrients and the conduction of food processing generally increases the bioavailability of micronutrients, antinutrients still possess important benefits, and food processing results in micronutrient loss. In general, biofortified foods with relatively higher micronutrient density have higher total absorption rates than nonbiofortified varieties. Thus, evidence supports the focus on efforts to breed plants with increased micronutrient concentrations in order to decrease the influence of inhibitors and to offset losses from processing.

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INTRODUCTION

Biofortification seeks to improve the nutrient density of staple food crops through conventional plant breeding, agronomic management, or genetic engineering. Currently, the most common micronutrients targeted are iron, zinc, and provitamin A carotenoids, due to the high prevalence of deficiencies of these micronutrients among children under the age of 5 years and women of child-bearing age in developing areas of Africa, Asia, and Latin America. Since a key issue in these areas is the low concentration of these micronutrients in the most commonly consumed foods, biofortification of staple food crops has been suggested as a way to help alleviate these deficiencies. Biofortification is particularly relevant in the current economy, when price increases for nonstaple foods further curtail dietary diversity and food and nutrition security among the poor. In principle, this strategy allows the population to grow and consume the same foods they are accustomed to eating while improving their micronutrient intake. Although concentrations of micronutrients in many of these biofortified crops will remain relatively low, staple foods are eaten in such large quantities in many at-risk populations that, over time, the micronutrients consumed in this manner can enhance micronutrient status and prevent deficiency.
Biofortification programs reach rural communities at risk for deficiency and thus are complementary to supplementation and fortification programs focused largely on urban and rural areas that are relatively easy to reach. Thus, biofortified staple foods may have the potential to substantially increase micronutrient intake and have a beneficial impact on micronutrient status and health, particularly among the most vulnerable.

The development and implementation of biofortification food programs is still in the early stages of growth. HarvestPlus, part of the Consultative Group on International Agriculture Research (CGIAR) Research Program on Agriculture for Nutrition and Health (A4NH), is currently supporting the research and dissemination of staple crops biofortified through conventional plant breeding.6–8 Prior to an intervention’s implementation, however, and in addition to agronomic traits, environmental factors, and variability in micronutrient concentrations, bioavailability is a major concern. Bioavailability, the amount of the nutrient that is accessible for utilization in normal physiological functions, metabolism, and storage, can be enhanced or inhibited by the presence of food components and food-processing techniques. Therefore, collaborative efforts are required between plant breeders, food scientists, and nutritionists to reach consensus on useful and meaningful target micronutrient levels. In order to properly estimate the minimum micronutrient concentrations that breeders must reach, as well as predict the ability of these interventions to be successful, the amount of the micronutrients present in the ready-to-eat portion of the plant and available for absorption must be investigated. The following article is a review of bioavailability studies conducted on conventionally bred biofortified staple crops being developed for large-scale food-based interventions and supported by HarvestPlus.

**BIOAVAILABILITY: DEFINING THE TERMS**

When discussing bioavailability studies, it is important to distinguish between the terms “bioavailability,” “bioaccessibility,” “bioconversion,” and “bioefficacy.” As mentioned previously, bioavailability is the fraction of an ingested nutrient that is available for utilization in normal physiological functions and/or for storage.9 Human and animal studies can provide information on bioavailability when the nutrient measured is in the blood (available for utilization), in animal tissue (storage), and, in the case of isotope studies, in excretions. Bioaccessibility is the amount of nutrient released from the food matrix and accessible for absorption.9,10 It is usually measured by in vitro methods such as simulated digestion and dialyzability. Although the Caco-2 cell model, an in vitro method, can measure the amount of nutrient that is taken up by the enterocytes, it is only considered to provide data on bioavailability if it is coupled with simulated digestion or dialyzability testing.11 Measured in humans and animals, bioconversion and bioefficacy are terms most often applied “most often” to provitamin A carotenoids.10,12 Bioconversion is defined as the fraction of the absorbed provitamin A carotenoids that is converted to retinol, while bioefficacy measures the efficiency at which provitamin A carotenoids are absorbed and converted to retinol and is typically assessed by measuring total-body vitamin A stores.12

**IN VITRO BIOACCESSIBILITY METHODS**

**Simulated digestion: solubility and micellerization**

In vitro simulated models simulate oral, gastric, and small intestine digestion by applying a series of digestive enzymes and pH conditions to assess the effects of the food matrix and processing on the digestive stability and bioaccessibility of a nutrient.13 While this method is most often combined with dialyzability and/or the Caco-2 cell model when evaluating iron and zinc absorption, it has been used alone extensively to evaluate the bioaccessibility of carotenoids from foods, meals, and supplements.14–17 This method can be modified to measure the presence of either carotenoids soluble in digestive fluids or micellarized carotenoids.11 The latter is preferred because the absorption of carotenoids requires transfer from the food matrix to emulsified oil droplets, which is followed by partitioning into mixed micelles during digestion in the small intestine, uptake into enterocytes, and incorporation and secretion in chylomicrons. One study has validated this technique against a human study.18 Although most in vitro results were in a range similar to results of the human study, spinach lutein was substantially more bioaccessible in vitro. Thus, simulated digestion studies cannot always be depended on as substitutes for human studies. Results of simulated digestion are difficult to compare between studies, since factors such as pH, incubation time, and centrifugation specifications often vary from study to study.

**Dialyzability**

The dialyzability model predicts the availability of nonheme iron and zinc following their release from the food matrix during simulated digestion.19 This model uses a two-stage simulated digestion process, along with a dialysis membrane with a defined molecular-weight...
The assumption in this model is that the membrane will filter out large-molecular-weight complexes likely to be unavailable for absorption while allowing more bioavailable small-molecular-weight complexes to penetrate the membrane. It is most useful for screening iron-containing foods for differences in micronutrient accessibility by taking into account diet composition, the chemical form of iron, and food-processing effects. Although one study by Chiplonkar et al. indicated that in vitro iron and zinc dialyzability strongly correlates with in vivo human data, concerns remain over the reliability of dialyzability data. For example, small polyphenol iron complexes that have low bioavailability are dialyzed, while the high-molecular-weight but bioavailable protein ferritin is excluded. These studies may not always predict the correct direction of response, and the magnitude of effect may not be the same as in human studies; moreover, they are incapable of testing the effect of high-dose consumption on absorption due to the lack of biological feedback mechanisms.

IN VITRO BIOAVAILABILITY METHODS

Caco-2 cell models

When combined with simulated digestion and/or dialyzability, Caco-2 cell models can assess in vitro bioavailability. Human intestinal Caco-2 cells are colonic carcinoma cells that display morphological and functional characteristics similar to those of differentiated epithelial cells from small intestinal mucosa. As the cells continue to mature upon culture, their morphological and biochemical characteristics more closely resemble those of enterocytes and show highly polarized columnar cells with tight junctions and desmosomes that result in the apical membrane separating from the basolateral membrane. The uptake of carotenoids is assessed by measuring the micellarized or solubilized compounds, while iron uptake is estimated through either ferritin formation or the use of a radioisotope (${}^{59}$Fe). Since there is no protein biomarker for zinc status like ferritin for iron status, zinc uptake is usually estimated by using a radioisotope (${}^{65}$Zn). While Caco-2 cells effectively predict the direction of response, it is still unclear whether they can predict the magnitude of response. In addition, there are many critical factors that must be controlled when using Caco-2 cells. Factors such as cell source, passage number, pH, makeup of incubation medium, cell maturation when supplemented, and composition and porosity of support material make comparison of results between laboratories difficult.

IN VIVO BIOAVAILABILITY AND BIOCONVERSION METHODS

Animal models

Animal models can provide useful information about in vivo bioavailability. For example, they allow for the dissection and analysis of individual tissues to provide a whole-body assessment of absorption. Although several species have been identified as appropriate models for estimating human absorption and metabolism, no animal model can exactly simulate the physiological response of the human. While rodent models can be suitable for assessing the bioavailability of zinc and carotenoids in some cases, they are a poor choice for assessing the bioavailability of iron. For example, rodents endogenously synthesize ascorbic acid, a promoter of iron absorption, possibly leading to an overestimation of iron bioavailability. For zinc, the rat pup model is the most appropriate model because young rats do not have intestinal phytase activity. Common models such as mice and rats have a high efficiency of cleaving provitamin A carotenoids in the intestine and therefore do not absorb carotenoids intact like humans do, rendering them inappropriate for assessing carotenoid bioavailability. Gerbils, however, absorb provitamin A carotenoids similarly to humans and are thus a suitable model.

In addition to the gerbil, the preruminant calf and ferrets also absorb provitamin A carotenoids intact and are suitable for use as animal models to assess the bioavailability and bioconversion of provitamin A. However, none of these animal models completely mimic carotenoid metabolism in humans. It also must be noted that the use of preruminant calves is costly and can only be used for short-term studies, and gerbils store large amounts of vitamin A in their liver, making it difficult to deplete stores; finally, ferrets have a relatively large amount of circulating retinyl esters in their blood.

Poultry animals may be a suitable model for measuring iron bioavailability due to their quick response to micronutrient deficiency, including low iron status. The use of recently hatched poultry is recommended because, at this early stage, broiler chicks are iron deficient and have a limited ability to synthesize vitamin C. Some physiological adaptation to iron deficiency may occur over time; therefore, the animals must be monitored for signs of anemia. Otherwise, it is difficult to discern differences in bioavailable iron between test samples. Piglets are also a suitable animal model for measuring iron.

Human studies

The use of radioactive and stable isotopes in human studies allows for the discrimination between the dose of
Bioavailability of iron, zinc, and provitamin A

micronutrient provided and endogenous forms of the micronutrient, allowing for a more accurate measurement of bioavailability. Micronutrients have been labeled both intrinsically and extrinsically. Intrinsic labeling is the biological incorporation of an isotope into the plant or the animal during its growth, while extrinsic labeling is the addition of an isotope to food prior to ingestion. It is important that the extrinsic labeling is validated by comparisons with intrinsic labeling experiments, which are reliable. It has been shown that extrinsic labeling can be used for nonheme iron (as in plant foods) and zinc absorption studies in humans. Numerous studies have also investigated carotenoid metabolism using 

\[ ^{14}\text{C}- \text{and} \ ^{13}\text{C}-\text{labeled compounds}. \]

Unfortunately, the high cost of these compounds and the labor-intensive procedures required have limited their use.

The total plasma carotenoid response can monitor changes in carotenoid concentrations in total plasma but can neither distinguish recently consumed from already-present carotenoids nor accurately assess bioconversion of provitamin A carotenoids to vitamin A. It also assumes that plasma clearance occurs at the same rate for all carotenoids. The preferred method of measuring carotenoid absorption and bioconversion is to isolate the triacylglycerol-rich lipoprotein plasma fraction, which contains newly absorbed carotenoids and their metabolites as well as newly formed retinoids. However, it is a labor-intensive method that requires collection of several postprandial plasma samples over the course of many hours. The separation of the triacylglycerol-rich plasma itself may be affected by human error, and separation of xanthophylls may be problematic because these compounds tend to be exchanged between lipoprotein particles.

**METHODS FOR ESTIMATING BIOAVAILABILITY**

**Mathematical models**

Algorithms are used to predict iron and zinc bioavailability. The usefulness of algorithms is highly dependent on accurate information on food intake and dietary absorption modifiers. Although iron absorption is most affected by iron status and the bioavailability of iron from the food consumed, numerous other host and dietary factors, such as recent intake and inflammation, contribute to its variability. There are several prediction equations that have been developed to estimate iron bioavailability. These equations were based either on human radioisotope studies measuring iron absorption or on food recalls and dietary records that were compared with iron status biomarkers from sample populations. Unfortunately, a single algorithm to predict iron absorp-

**USEFULNESS OF DIFFERENT METHODS**

Human studies provide the most applicable results because they are capable of considering host factors, disease states, and physiological changes during digestion. Therefore, such results can be interpreted more directly and used to assess true absorption of nutrients from foods. Although experimental animals can have physiological responses similar to those in humans and can allow for the analysis of specific tissues, no animal model can be considered to digest and absorb nutrients exactly the same as humans do. Even though algorithms are based on human data, they are merely estimations and only factor a limited number of the influencers of absorption; thus, they are only as reliable as the inputs they include. They are most useful for providing broad estimations and for categorizing foods. Finally, in vitro studies cannot emulate all of the physiological and metabolic responses and thus are not useful for providing data directly applicable to humans. In addition, comparisons between different studies with regard to percentage absorption results are unreliable because there are many variables that are difficult to replicate, and no standardized method of conducting these studies exists. Since their applicability to humans is limited, in vitro studies should be viewed as a means of screening, ranking, and categorizing cultivars and foods, large numbers of genetic variants, food-processing effects, and influencers of absorption and directing attention to factors that may deserve further investigation in humans.

So that they may be clearly distinguished, human studies have been separated from other absorption studies when providing study synopses in this review. The similarities and differences in results are discussed, as are key findings. In addition, the bioavailability and bioaccessibility data for each study listed in the tables are grouped together by the type of study performed to enable easier within-study comparisons.

**BIOFORTIFICATION**

Due to the lack of genetic variation in adapted germplasm, the most common way of biofortifying crops is by transferring micronutrient density present in
unadapted sources into high-yielding competitive genetic backgrounds by conventional cross-breeding techniques. In addition, agronomic attributes and end-use quality traits must always be considered when developing trait packages attractive to farmers to trigger adoption. Once a high-micronutrient, high-yielding variety is developed at international breeding centers and transferred to target countries, breeding for local adaptation, in particular, local biotic and abiotic production constraints and specific end-use quality requirements or distinct market classes, may be necessary.

Agronomic biofortification is a strategy that can provide a temporary increase in micronutrients through the application of fertilizers. Micronutrient fertilizers can increase levels of zinc, nickel, iodine, copper, molybdenum, and selenium in varying degrees in the edible portion of certain plants. This is especially effective in the case of foliar-applied zinc, used to increase zinc concentrations in wheat grain.

Transgenic approaches are the only feasible option to increase the micronutrient concentration when attempting biofortification with a micronutrient that does not naturally exist in the crop. Further, a transgenic approach may facilitate the simultaneous incorporation of genetic systems to enhance micronutrient concentration, decrease antinutrients, and increase promoters of bioavailability. Genetic modification for biofortification purposes has been performed on numerous staple crops, including rice, cassava, maize, and wheat. The present review is limited to studies that used biofortification through conventional breeding.

Breeding for bioavailability

Methods and considerations for biofortifying plants with micronutrients vary, depending on the type of plant and the micronutrient. For minerals such as iron and zinc, the micronutrient is transferred from the soil to the plant to the human intact and is not transformed during uptake and metabolism. Thus, there is a high genotype × environment (G×E) interaction that occurs with iron and zinc. Several genes are involved in the uptake, translocation, and loading as these nutrients are transferred from soil to seed. In contrast, provitamin A carotenoids are synthesized by the plant and have substantially fewer genes inherited with each cross. Breeding to increase bioavailability has been one of the approaches used and can be done by manipulating plant structures or increasing promoters and/or decreasing inhibitors such as antinutrients.

Reduction of antinutrients

Current strategies applied by plant breeders aim to decrease antinutrients such as phytates, oxalates, and polyphenols in food crops. These compounds can chelate iron and zinc to form insoluble complexes in the gastrointestinal tract that, consequently, reduce their bioavailabilities. However, they also possess benefits that preclude breeders from aiming to remove them entirely. For example, these antinutrients are integral to plant metabolism and to resistance to pests, pathogens, and abiotic stress. Furthermore, some so-called antinutrients, such as phytic acid (inositol hexaphosphate [IP6] and other inositol phosphates), may provide health benefits for humans, including anticarcinogenic effects and decreased risk of cardiovascular disease and diabetes. The impact of antinutrients on provitamin A carotenoids is unclear but is not a major concern.

Enhancement of promoters

Breeding to increase bioavailability promoters is also being explored. Small changes in the concentrations of these compounds may result in a comparatively large improvement in bioavailability. Few promoters have been identified for iron and zinc. Phytoferritin may be the most promising promoter of iron absorption, depending on the extent to which it effectively protects iron from binding to phytates. However, knowledge of its genetic variability is limited, and thus it is still unknown whether phytoferritin concentrations can be increased in foods. Another promising promoter is nondigestible carbohydrates or prebiotics, such as fructans, which promote the growth of beneficial “probiotic” bacteria in the hind gut that in turn may decrease inflammation and limit the growth of pathogenic bacteria. The growth of some types of such bacteria has yet to be shown to improve iron and zinc absorption. Activation of endogenous phytases can increase both iron and zinc bioaccessibility. Endogenous phytases are capable of hydrolyzing hexa- and pentaphosphates to decrease inhibitory inositol phosphates that bind to minerals.

Other strategies

Although promoters such as ascorbic acid and cysteine can be increased through breeding, they are highly susceptible to degradation during processing and thus unlikely to have a significant impact on increasing iron bioavailability. There are no known promoters of carotenoid absorption that could potentially be increased by breeding. However, breeders could select for genotypes that show higher retention of carotenoids (i.e., lower carotenoid degradation) after processing and, in particular, after storage, which, along with bioavailability, is of great concern.
**BIOFORTIFIED STAPLE FOOD CROPS**

The primary crops currently being biofortified by conventional breeding include some of the most important staple crops in the world: maize (Zea mays L.), rice (Oryza sativa L.), wheat (Triticum spp.), beans (Phaseolus vulgaris L.), cassava (Manihot esculenta Crantz), sweet potatoes (Ipomoea batatas L.), and pearl millet (Pennisetum glaucum). Due to the absence or low concentrations of certain micronutrients or the lack of variability between cultivars, not all micronutrients are suitable for biofortification in every staple crop. Thus, programs such as HarvestPlus have been tasked with identifying which micronutrients can be enhanced by biofortification in each staple crop.

Maize, sweet potatoes, and cassava are being biofortified to contain higher concentrations of provitamin A carotenoids. Maize is a dietary staple for over 200 million people worldwide, providing approximately 20% of the world’s calories. Approximately 70 million people obtain at least 500 calories a day from cassava. Furthermore, cassava consumption is increasing rapidly, since cassava maintains its nutritional value during times of drought, is flood resistant, and can be grown year-round. Forty of 82 developing countries rank sweet potatoes as one of their five most important food crops. Biofortification of sweet potatoes represents an unusual case, since there are already many varieties of sweet potatoes that contain exceptionally high concentrations of provitamin A carotenoids. Although sweet potatoes are already a staple food in many vitamin-A-deficient regions such as sub-Saharan Africa and Southeast Asia, the white-yellow varieties commonly consumed have, unfortunately, a far lower concentration of provitamin A carotenoids than the orange-fleshed varieties.

Wheat, common beans, rice, and pearl millet are being biofortified to contain higher concentrations of iron and/or zinc. Rice is a major staple food in developing Asian countries, where it provides 40–70% of total daily calories. Wheat is typically the second- or third-most-produced cereal in the world on an annual basis. The common bean is a major staple food in Central and South America and Eastern Africa, consumed by over 300 million people. Pearl millet is a widely consumed crop in the semiarid tropics of India and sub-Saharan Africa, accounting for greater than 50% of total cereal grain consumption in some Indian communities and 26% of average per capita cereal grain consumption in western Africa.

**IRON BIOFORTIFICATION**

Iron is an integral component of hemoglobin and myoglobin as well as a constituent of heme and nonheme enzymes that participate in oxidation-reduction reactions. In developing countries such as Africa and Southeast Asia, the prevalence of iron-deficiency anemia is approximately 50% in pregnant women and school-aged children. The World Health Organization estimates that 1.6 (95%CI, 1.50–1.74) billion people have anemia, the most common consequence of iron deficiency. Iron-deficiency anemia has been shown to cause cognitive and physical impairment in children and to affect the physical performance of adults.

**Bioavailability factors**

Unlike the heme iron found in animal products, nonheme iron in plants generally has low bioavailability. Absorption of nonheme iron can be improved or impeded by the binding of iron to compounds that create complexes such as chelators and ligands. When iron forms a loosely bound complex, it retains its solubility and can be released and absorbed by the intestinal cell. However, if strongly bound to a complex, it may become insoluble, thereby inhibiting its absorption and increasing its likelihood to be excreted.

Dietary components that promote iron absorption include organic acids such as ascorbic and citric acid, meat, fish, and poultry, along with their digestion products, and mucin. Organic acids such as ascorbic and citric acid reduce iron to its ferrous state and produce a soluble iron chelate in the small intestine that improves absorption. The enhancement of absorption from meat, fish, and poultry is believed to be due to digestion products that contain high amounts of cysteine peptides that can increase the absorption of iron and of amino acids that chelate iron and are absorbed intact by amino acid carrier proteins. Mucin binds ferric iron when present at the low pH of the stomach and subsequently will confer solubility to the iron when it is exposed to the higher pH of the small intestine.

Dietary components that inhibit iron absorption include polyphenols, oxalic acid, phytates, phosvitin, and some nutrients, including calcium, calcium phosphate, and zinc. Polyphenols, such as tannins in tea and coffee, can dramatically reduce iron absorption. Phytates and oxalates bind several minerals, including iron and zinc, to form complexes that are insoluble and inefficiently absorbed. Phytates, including IP6 and inositol pentaphosphate (IP5), are commonly found in cereals, bran, and legumes, while oxalates are common in chocolate, tea, and spinach. Iron and zinc can inhibit each other’s absorption, but studies indicate that this negative interaction only occurs when they are both in the same solution, and not when they are in the same meal.

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Food processing often increases iron bioavailability by reducing antinutrients. In addition, fermentation can increase the concentration of promoters such as citric and lactic acid.

STAPLE FOOD CROPS BIOFORTIFIED WITH IRON

Rice

One study conducted in humans indicates that rice has a fractional iron absorption of 17.2% and that its consumption can help increase or maintain iron stores (Table 1). In a 9-month double-blind dietary intervention in Filipino women by Haas et al., the experimental group consumed 1.79 mg of iron per day in steamed iron-biofortified rice, while the control group consumed 0.37 mg of iron per day in a local rice variety. From the data acquired in this study, Beard et al. calculated a 17.2% median efficiency of absorption based on gain in body iron combined with daily iron requirement estimates. Further analysis of the experimental group revealed that women at the 25th percentile of baseline body iron at the beginning of the study had a 22 μmol/kg increase in body iron, while those with higher initial stores maintained them throughout the study. Thus, consumption of biofortified rice with a relatively low concentration of iron may still have a positive effect on iron status as compared with consumption of unfortified rice.

As an addition to the Haas et al. study mentioned previously, the fractional absorption results were compared with six iron absorption prediction estimates. The comparison indicated significant differences between the results of different algorithms as well as the results of the human study by Haas et al. Specifically, the iron absorption prediction estimates ranged between 3.0% and 8.0%. The authors suspected that the differences were likely due to the use of point estimates based on a single isotope-labeled meal to calculate prediction estimates. As a result, they cannot account for iron requirements over a long period of time. In contrast, Haas et al. used weighed food intakes over a period of several months. Although this method of assessing dietary intake is very accurate, it is only practical in highly controlled settings and thus is seldom used. In addition, multiple indicators of iron status were used.

Beans

Two human studies provide support that phytic acid content is the most important factor influencing iron bioavailability in beans. The impact of polyphenols on iron absorption may be minimal, but the results thus far have been inconsistent. Fractional absorption of iron was estimated to be between 2.6% and 7.4%, and it decreased with higher intake.

Two human studies by Petry et al. investigated the inhibitory effect of polyphenols on iron bioavailability. The first study was conducted in two sets of triple stable isotope studies in women to test the bioavailability of iron from common beans using single meals. The first three studies tested the effect of a variety of polyphenol concentrations (20, 50, and 200 mg) in bean hulls in a reference meal on iron bioavailability in order to investigate the influence of polyphenol concentrations relative to phytic acid. Three other experiments were designed to test changes in iron bioavailability in the presence or absence of polyphenols and/or phytic acid in bean porridge. The presence of varying concentrations of polyphenols provided conflicting results. However, similar to previous single-meal studies on the bioavailability of iron from common beans, there was an increase in bioavailability with dephytinization. For example, the increase in iron absorption from whole beans and dephytinized bean meal was 2.4% and 3.5%, respectively. Removing both phytic acid and polyphenols increased iron absorption by 2.6-fold (P < 0.001), and removing the hulls along with phytates increased iron absorption by 2-fold over a dephytinized meal alone. Since iron absorption was lower when hulls were removed, the authors hypothesized that another compound in hulls improves the absorption of iron, but only when phytate is present. Since results for polyphenols are inconsistent, a focus on lowering the phytic acid content of beans may have a more predictive effect on the bioavailability of iron from beans.

The second study by Petry et al. focused on polyphenol inhibition and the potential to provide iron-biofortified beans to Rwandese women with low iron status. The first two experiments evaluated the impact of low- and high-polyphenol meals with similar concentrations of iron and phytic acid using both double- and multiple-meal designs, while the third experiment assessed iron absorption from low- and high-iron beans with similar polyphenol and phytic acid content using multiple-meal designs. Beans were boiled and homogenized. Fractional absorption of iron was 28% lower from the high- versus the low-polyphenol meal (P < 0.01) in the double-meal experiment. Interestingly, this dramatic difference in iron absorption was not observed when the same meals were prepared as multiple meals combined with rice or potatoes twice per day over 5 days instead of two bean porridge meals on one single day. In fact, there was no significant difference in iron absorption in this particular experiment. The lack of impact of polyphenols when consumed with multiple meals may be a result of the dilution of the effect of polyphenols due to other food.
<table>
<thead>
<tr>
<th>Crop</th>
<th>Meal or processing method</th>
<th>Group</th>
<th>Model</th>
<th>Iron bioavailability (% absorption)</th>
<th>Final iron content (mg/kg DW)</th>
<th>Phytate content (mg/kg DW)</th>
<th>Phytate:Iron molar ratio (X:1)</th>
<th>No. of cultivars</th>
<th>Notes</th>
<th>Reference</th>
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<td>Beans</td>
<td>Stew</td>
<td>Control</td>
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<td>45.8</td>
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<td>with dialyzability</td>
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<td>RM + bean hulls (20 mg PP/RM)</td>
<td>Human isotope study</td>
<td>13.9/14.2</td>
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<td>NA</td>
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<td>4.7/4.6 mg iron, 0/0 mg phytate</td>
<td>Petry et al. (2010)</td>
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<td>4.8/4.6 mg iron, 0/0 mg phytate</td>
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<td>1</td>
<td>6.1/6.0 mg iron, 0/0 mg phytate</td>
<td></td>
</tr>
<tr>
<td>Beans</td>
<td>Boiled</td>
<td>High PP bean</td>
<td>Human isotope study</td>
<td>3.4 (double meal)</td>
<td>52</td>
<td>8,500</td>
<td>13.8</td>
<td>1</td>
<td></td>
<td>Petry et al. (2012)</td>
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<tr>
<td></td>
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<td>Low PP bean</td>
<td></td>
<td>4.7 (double meal)</td>
<td>71</td>
<td>8,700</td>
<td>10.4</td>
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<td></td>
<td></td>
<td>High PP bean + rice/potato</td>
<td></td>
<td>7.0 (multiple meal)</td>
<td>66</td>
<td>7,800</td>
<td>10.0</td>
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<td></td>
<td></td>
<td>Low PP bean + rice/potato</td>
<td></td>
<td>7.4 (multiple meal)</td>
<td>73</td>
<td>8,500</td>
<td>9.9</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>High iron bean + rice/potato</td>
<td></td>
<td>3.8 (multiple meal)</td>
<td>52</td>
<td>8,500</td>
<td>13.8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal iron bean + rice/potato</td>
<td></td>
<td>6.3 (multiple meal)</td>
<td>92</td>
<td>13,900</td>
<td>12.8</td>
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</tr>
<tr>
<td>Rice</td>
<td>Steamed</td>
<td>Biofortified</td>
<td>Mathematical model</td>
<td>17.2</td>
<td>3.2</td>
<td>NA</td>
<td>50</td>
<td>1</td>
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<td>Beard et al. (2007)</td>
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<tr>
<td>Pear millet</td>
<td>Flour</td>
<td>Control</td>
<td>Human isotope study</td>
<td>7.5</td>
<td>88</td>
<td>8,520</td>
<td>8.2</td>
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<td>Cercamondi et al. (2013)</td>
</tr>
<tr>
<td>Pear millet</td>
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<td>Biofortified</td>
<td></td>
<td>7.5</td>
<td>25</td>
<td>6,530</td>
<td>22.1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DW, dry weight; NA, data not available; PP, polyphenols; RM, reference meal.
* The Petry et al. (2010) study design included six iron isotope absorption experiments, each including two meals. The data for each meal are separated by a forward slash.
components in potatoes or rice that may enhance iron absorption. In the third experiment, fractional absorption of iron was 40% higher ($P < 0.001$) from multiple meals of a typical variety of red beans than from biofortified black beans. This result supports previous studies showing downregulation of iron absorption in response to higher intakes. However, in this experiment, the authors suggested that the inhibitory effect of phytic acid was likely to be the major reason for the lower fractional and total iron absorption, with polyphenols having a small effect relative to phytic acid. This result is highly informative for biofortification bean breeding efforts, since it indicates that improvements to date in iron concentrations of biofortified varieties could be insufficient to improve short-term iron absorption over that currently achievable with nonbiofortified varieties.

An animal study and an in vitro digestion dialyzability study observed high iron absorption from biofortified beans, at 20.9% and 9.5%, respectively. Animal studies have yielded mixed results as to the increased bioavailability of iron from consumption of biofortified beans versus nonbiofortified beans. An in vitro study showed polyphenols to have a greater negative impact than phytates on iron bioavailability.

In the first study, Tako et al. assessed the ability of iron-biofortified and standard black beans consumed in a maize-based diet to increase hemoglobin synthesis in a 5-week feeding study using iron-deficient piglets. At the conclusion of the study, the hemoglobin repletion efficiency, used as a measure of iron bioavailability, did not differ between groups, indicating that although the biofortified beans contained higher concentrations of iron, net iron absorption from these beans was the same as that from standard beans. Given the similar phytate:iron molar ratios and total phenol content of both types of beans and the significant contribution of iron by nonbiofortified beans, this lack of a difference was not surprising.

In another study, Tako et al. utilized a combination of the in vitro Caco-2 cell model and a poultry (Gallus gallus) animal model to evaluate the bioavailability of iron from high-iron biofortified red mottled beans versus low-iron standard red mottled beans over the course of 4 weeks. Hemoglobin maintenance efficiency, a measure of iron bioavailability, was significantly higher in animals fed the biofortified beans than in animals fed the standard beans at days 14 and 21 of the experiment. There were no significant differences in liver ferritin or iron concentrations between the two groups. Contrary to these results, the concurrent in vitro digestion/Caco-2 cell model showed a significant increase ($P < 0.05$) in ferritin. However, in vitro studies supported that net iron absorption was higher from the higher-iron bean diet than from the lower-iron bean diet.

A separate study yielded results that conflicted with those of Tako et al. In a study by Pachon et al. using in vitro dialyzability, iron bioaccessibility was not significantly different between biofortified and conventionally bred beans. This is likely due to the two bean varieties having nearly equal iron concentrations and high phytate:iron molar ratios.

A study by Ariza-Nieto et al. assessed iron bioavailability from eight common bean genotypes (Phaseolus vulgaris) utilizing an in vitro digestion/Caco-2 cell model. In contrast to the results of the human study by Petry et al., polyphenols were more inhibitory than phytates. Significant improvements in iron in vitro bioavailability were observed when the polyphenol-containing seed coats were removed.

**Pearl millet**

Two studies performed in humans provide bioavailability data for biofortified pearl millet and estimated a fractional absorption of 7.5% and 9.0%, respectively. Both studies indicated an improvement in total iron absorption but a lack of increased absorption percentage with a biofortified meal versus a standard meal.

A study in Beninese women investigated fractional and total absorption of iron from multiple meals based on iron-biofortified millet, regular millet, or postharvest iron-fortified millet paste made from flour. Meals were fed over a period of 5 days, with iron absorption measured as erythrocyte incorporation of stable isotopes 2 weeks later. The concentration of iron was 0.88 μg/g in the biofortified meal and 0.25 μg/g in regular millet. Mean fractional absorption from the iron-biofortified millet meal was not significantly different than that from the regular millet meal, with both meals having a fractional absorption of 7.5%. However, the amount of iron absorbed was twice as high from the biofortified meal ($P < 0.0001$). Iron absorption from the postharvest iron-fortified meal was significantly higher than that from both the regular and the biofortified meals ($P < 0.05%$), with a fractional absorption of 10.4%. Although biofortified millet contained increased phytic acid concentrations, the phytate:iron ratio actually decreased from 22.1 in regular millet to 8.2:1 in the biofortified millet due to the increased iron content of the former. The very low phytate:iron ratio in the fortified millet (6:1), in accordance with previous reports showing that a ratio of less than 6:1 improves iron absorption, may explain why this meal was better absorbed.

Another study of the fractional absorption of iron from pearl millet, which utilized stable isotope extrinsic labeling techniques, was performed in iron-deficient children in India. Fractional absorption efficiencies of 9.0% for biofortified and 6.0% for regular millet flour were not
significantly different. However, the total amount of absorbed iron was significantly higher. Thus, it is promising to note that the 0.7 mg of iron absorbed per day from biofortified millet surpassed the daily iron intake requirement (0.54 mg/d) for their age group.89

ZINC BIOFORTIFICATION

Zinc, often the most concentrated trace element in cells, is an essential mineral for cell development, gene expression, and replication.90 The risk of zinc deficiency is a worldwide public health problem, with an estimated 17.3% of the population at risk of inadequate zinc intake.102 Research indicates zinc deficiency can limit linear growth, weaken the immune response, and leave those afflicted more susceptible to infection. In fact, it may increase morbidity and mortality from infections such as diarrhea, acute respiratory tract infections, and malaria.103

Bioavailability factors

Dietary factors that improve zinc absorption include amino acid ligands such as histidine and cysteine, products of protein digestion such as tripeptides, and endogenous ligands such as citric acid. Inhibitors of zinc absorption are similar to those of iron. These include phytate, oxalate, polyphenols, fibers, and some other cations. Zinc derived from animal sources is much more available than that from plant sources, as plants contain large amounts of inhibitors, as mentioned above.104–106 Plant-source zinc also forms insoluble complexes at pH 6, a pH common for foods.106

Interestingly, food-processing methods can have differing effects on absorption. For example, heat treatment can create zinc complexes resistant to hydrolysis and absorption. Maillard reactions can also inhibit zinc absorption.93 On the other hand, processing techniques such as soaking and fermentation partially hydrolyze phytate into analogous metabolites with less zinc-binding capacity and a lower inhibitory effect.107

STAPLE FOOD CROPS BIOFORTIFIED WITH ZINC

Rice

No zinc bioavailability studies using biofortified varieties of rice in humans have been published; however, much can be learned from a human study that estimated a fractional absorption of 20.1% from a high-zinc variety of rice (Table 2).108

In a study in Bangladeshi children by Islam et al.,108 a dual-isotope tracer ratio technique was used to calculate fractional absorption and total absorbed zinc from high-zinc rice bred for high altitude. Although zinc intake from the conventional rice-based diet was 1 mg less than intake from the high-zinc diet, the total absorbed zinc from these diets was not significantly different. This was due to lower fractional absorption (20.1%) and estimated phytate content in the high-zinc rice as compared with the conventional rice (25.1% fractional absorption). It was concluded that the high-zinc rice likely would have to have contained 1.2 mg more zinc than the conventional rice for a difference in total absorbable zinc to be detectable, and that rice cultivars with higher zinc and/or lower phytate must be developed in order to benefit young children. However, this conclusion may not be applicable to adults, since they are capable of consuming larger amounts of rice. Thus, studies on adult subjects who consume biofortified varieties will be necessary to make a conclusion for this age group.

The absolute percentage of zinc absorption from high-zinc rice predicted by an algorithm was inaccurate.65 Results of in vitro and rat pup studies differed from human and algorithm data in that they did not show a decreased percentage of zinc absorption with a higher zinc intake from biofortified rice.37

In the study by Islam et al.108 described previously, the absorption of zinc from rice was predicted using an algorithm by Miller et al.65 The algorithm predicted zinc absorption was 35.4% for conventional rice and 30.1% for high-zinc rice. Of course, although zinc absorption prediction estimates have been validated,55 they cannot be considered a replacement for human studies.

Both a Caco-2 cell model and a rat pup animal model were used to assess the bioavailability of zinc from biofortified undermilled and polished rice.37 No significant differences in bioavailability were observed between the undermilled versus the polished rice. Zinc bioavailability results from the Caco-2 cell model were highly correlated with those from the rat pup model. This is encouraging, since results from rat pups have previously been found to be correlated with human absorption studies. However, it must be noted that the absolute numbers were different. Since phytate:zinc molar ratios of the rice breeds studied ranged from 19 to 52, it is not surprising that no effect of phytate concentration on zinc absorption was observed in the study. Past studies indicate that the inhibitory effect is only linear for phytate:zinc molar ratios between 2.9 and 11.4.109 while higher molar ratios do not show a further effect.13 In an experiment performed in this study, there were significant decreases in zinc uptake with phytate:zinc molar ratios between 2.5 and 20. However, there were no significant differences between ratios of 30, 40, and 50 versus 20. The net uptake of zinc from the biofortified variety versus the conventional varieties was proportional to the
Table 2: Zinc bioavailability studies.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Meal or processing method</th>
<th>Group</th>
<th>Model</th>
<th>Zinc bioavailability (% absorption)</th>
<th>Final zinc content (mg/kg DW)</th>
<th>Phytate content (mg/kg DW)</th>
<th>Phytate:zinc molar ratio (X:1)</th>
<th>No. of cultivars</th>
<th>Notes</th>
<th>Data type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>Polished</td>
<td>Control</td>
<td>Rat pups</td>
<td>40–59</td>
<td>14.5–18.8</td>
<td>5,098–8,204</td>
<td>34.5–45.8</td>
<td>4</td>
<td></td>
<td>Ratio calculated</td>
<td>Jou et al. (2012)</td>
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<tr>
<td></td>
<td></td>
<td>Biofortified</td>
<td></td>
<td>42</td>
<td>35.5</td>
<td>6,907</td>
<td>19.2</td>
<td>1</td>
<td></td>
<td>Ratio calculated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Undermilled</td>
<td>Control</td>
<td></td>
<td>36–47</td>
<td>17.9–21.9</td>
<td>8,141–11,186</td>
<td>44.6–51.9</td>
<td>4</td>
<td></td>
<td>Ratio calculated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biofortified</td>
<td></td>
<td>40</td>
<td>42.5</td>
<td>12,932</td>
<td>29.9</td>
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<td></td>
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</tr>
<tr>
<td>Rice</td>
<td>Polished</td>
<td>Control</td>
<td>Human isotope study</td>
<td>25.1</td>
<td>15.8</td>
<td>3,190</td>
<td>20</td>
<td>1</td>
<td>Used Miller et al. (2007) model</td>
<td>Islam et al. (2013)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>High-zinc</td>
<td></td>
<td>20.1</td>
<td>23.9</td>
<td>5,300</td>
<td>22</td>
<td>1</td>
<td></td>
<td>Ratio calculated</td>
<td></td>
</tr>
<tr>
<td>Wheat Flour</td>
<td>Control</td>
<td>Mathematical model</td>
<td></td>
<td>35.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Used Miller et al. (2007) model</td>
<td>N.F. Krebs MD, (written communication, 2007)</td>
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<tr>
<td></td>
<td></td>
<td>High-zinc</td>
<td></td>
<td>30.1</td>
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<td></td>
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<tr>
<td></td>
<td>Biofortified</td>
<td>Math model</td>
<td></td>
<td>48.6 (95% extraction)</td>
<td>23.3 (95% extraction)</td>
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<td>47.5 (95% extraction)</td>
<td>40.0 (95% extraction)</td>
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<tr>
<td>Wheat Flour</td>
<td>Control</td>
<td>Human isotope study</td>
<td></td>
<td>20 (95% extraction)</td>
<td>23</td>
<td>9,000</td>
<td>38.8</td>
<td>8</td>
<td>Rosado et al. (2009)</td>
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<td></td>
<td>Biofortified</td>
<td></td>
<td>15 (95% extraction)</td>
<td>40.5</td>
<td>9,300</td>
<td>22.8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td>38 (80% extraction)</td>
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<td>3,400</td>
<td>23.4</td>
<td>8</td>
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<tr>
<td>Pearl millet</td>
<td>Flour</td>
<td>Control</td>
<td>Human isotope study</td>
<td>20</td>
<td>43.7</td>
<td>10,300</td>
<td>23.3</td>
<td>1</td>
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<td></td>
<td>Kodkany et al. (2013)</td>
</tr>
<tr>
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<td>Biofortified</td>
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<td>17</td>
<td>84.1</td>
<td>7,500</td>
<td>8.8</td>
<td>1</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: DW, dry weight.

* Ranges are given when different cultivars were tested.

* All data were disclosed in the article unless otherwise noted. In some cases, the phytate:zinc molar ratio was not disclosed and had to be calculated.
zinc content of the latter, with zinc absorption being significantly higher for the biofortified variety in both the in vitro and rat pup models. Thus, increased zinc concentration did not result in compromised zinc bioavailability.

**Wheat**

The only human study of zinc bioavailability from biofortified wheat indicates that overall zinc absorption may be improved with biofortified wheat as compared with standard varieties.110 Rosado et al.110 compared zinc absorption from 95% and 80% extracted high-zinc biofortified wheat using a dual isotope tracer ratio technique in humans. Both extraction rates resulted in a similar reduction in zinc and phytate contents, suggesting that the benefits of high-zinc wheat are not negatively affected by milling. Although fractional zinc absorption was slightly lower from the biofortified versus the control variety of wheat, the quantity of zinc absorbed from wheat in women was enhanced by biofortification. At two different extraction levels (95% and 80%), biofortified flours contributed 0.5 mg more absorbed zinc than flours from conventional wheat.

Interestingly, a zinc absorption model predicted a nearly identical amount of zinc absorbed as the human study.110 A second trivariate model from a different study predicted the ability of biofortified wheat to meet zinc physiological requirements (N.F. Krebs, MD, written communication, 2007).

The trivariate zinc absorption model used in the Rosado et al.110 study yielded iron absorption prediction estimates nearly equal to those in the human study. Although this congruency is promising, many more trivariate models compared directly with results obtained in human studies will need to be established before they can be considered accurate.

In another study, Krebs et al. (N.F. Krebs, MD, written communication, 2007) evaluated the effect of gut length adjustment on the fit of zinc absorption and dietary zinc and phytate for toddlers to a trivariate model for zinc absorption as a function of the same dietary data for adults.65,111,112 Subsequently, they used this model to evaluate zinc absorption from high-zinc biofortified wheat in young children. At 95% extracted wheat, and following consumption of 1.2 mg of zinc from high-zinc biofortified wheat, 0.57 mg was predicted to be absorbed. At 80% extracted wheat, and following consumption of 0.7 mg of zinc from high-zinc biofortified wheat, 0.35 mg was predicted to be absorbed. An important conclusion was that the high-zinc wheat with high extraction is capable of meeting physiological zinc requirements and that a minimum of 30 g of high-zinc biofortified wheat flour is required for zinc absorption measurements in children.

**Beans**

Despite the potentially significant contribution of beans to the daily intake of zinc, there are no published studies available that have tested the bioaccessibility or the bioavailability of zinc from biofortified beans.

**Pearl millet**

The bioavailability of zinc in biofortified pearl millet has been investigated in only one study, which indicated a fractional absorption of 17%.89

A previously mentioned study in 2-year-old children in India measured fractional absorption of not only iron but also zinc.89 Zinc consumption was 5.8 mg/d with biofortified pearl millet and 3.3 mg/d with regular pearl millet. Using stable isotope extrinsic labeling of zinc, fractional absorption from the biofortified and regular millet was found to be 17% and 20%, respectively. Although this fractional absorption was less than expected, the amount of zinc absorbed was higher from the biofortified than from the regular millet. As a promising note, the zinc absorbed from the biofortified millet exceeded the estimated physiological zinc requirement for this age group.

**PROVITAMIN A BIOFORTIFICATION**

Vitamin A plays a key role in many biological processes in the body, including growth, vision, reproduction, immunity, and cellular differentiation and proliferation.113 Vitamin A deficiency affects an estimated 190 million preschool-aged children and 19.1 million pregnant women in developing countries.86 Xerophthalmia, the leading cause of preventable pediatric blindness in developing countries, afflicts 5 million preschool-aged children.113 It is also prevalent in women of childbearing age and may increase the risk of maternal morbidity and mortality in susceptible populations.113

**Bioavailability factors**

Dietary factors that affect provitamin A carotenoid bioavailability include the food matrix, food processing, and others.114 Perhaps the most influential factor for carotenoid bioavailability is the food matrix. β-carotene supplements in oil and natural food sources such as red palm oil have the highest bioavailability.115 Bioavailability of carotenoids in plant matrices is a much more complex issue. Carotenoids in green leafy vegetables are located in chloroplasts and are bound to pigment-protein complexes that result in low bioavailability.116 On the other hand, carotenoids in orange, yellow, or red plants are in crystalline form within oil droplets of membranes of large proteins, resulting in higher bioavailability.117
Food-processing techniques such as heating and mechanical homogenization increase bioavailability by breaking cell walls, hydrolyzing carotenoid-protein complexes and decreasing particle size. Unfortunately, extreme processing techniques such as prolonged boiling at high heat, roasting, and frying can cause large losses of carotenoids and isomerization. However, mild techniques such as low-heat cooking, short-term boiling, soaking, and chopping can enhance bioavailability, with minimal carotenoid loss.

The structure of the carotenoid may affect its absorption. It is believed that the presence of more polar xanthophylls, such as β-cryptoxanthin, on the outer surface of micelles results in greater absorption of carotenoids than carotenes. Evidence of carotenoid esters is mixed, since studies suggest equivalent uptake of esterified and nonesterified carotenoids. Cis isomers are generally believed to be more polar and soluble than their all-trans forms.

Dietary factors can also act to enhance or inhibit carotenoid absorption. Dietary fat can increase carotenoid absorption by initiating the excretion of bile salts, which form micelles that make carotenoids more soluble. Studies indicate that only 3–6 g of fat is required for optimal absorption. The type of fat may also influence absorption.

Key inhibitors of carotenoid absorption are dietary fiber, including lignan, pectin, gels, cellulose, and bran. For example, fiber inhibits absorption by entrapping carotenoids and by maintaining bile salts and cholesterol in a gel phase rather than forming micelles that facilitate carotenoid absorption.

STAPLE FOOD CROPS BIOFORTIFIED WITH PROVITAMIN A CAROTENOIDS

It should be noted that most of the provitamin A carotenoid studies used similar study designs and evaluated similar outcomes. Thus, unlike iron and zinc, the results of the studies in the following section are often combined rather than discussed individually. However, in parallel with the rest of this review, comparisons of nonhuman studies with regard to percentage absorption are made within studies and not between studies.

Orange-fleshed sweet potatoes

Studies with biofortified orange-fleshed sweet potato included one human study, one animal study, and multiple in vitro studies (Table 3). A study in Bangladeshi women who consumed biofortified orange-fleshed sweet potato or white-fleshed sweet potato reported an increase in total-body vitamin A in the group who consumed fried biofortified sweet potatoes, but this increase was not significant when compared with the group who consumed white-fleshed sweet potatoes. Total-body vitamin A in the group who consumed boiled biofortified orange-fleshed sweet potatoes increased to concentrations equivalent to those observed in the white-fleshed sweet potato group.

A study assessing biofortified sweet potato using Mongolian gerbils demonstrated that the efficiency of bioconversion of β-carotene to retinol was dependent on the fat content of the diet. For a high-fat diet, the bioconversion was (3.5:1) as compared with the lower-fat meal (6.5:1) and the no-fat meal (6.7:1). In the same study, the influence of pectin on absorption was also investigated. Although pectin in the orange-fleshed sweet potatoes was confirmed to be the most highly concentrated soluble fiber, it did not negatively affect bioconversion.

The bioaccessibility of β-carotene in orange-fleshed sweet potato is highly variable, with estimates ranging from 0.6% to 73%. As noted in the gerbil study, differences in the fat content of the food products or between the different assays were partially responsible for this variation. A study by Mills et al. reported an increased bioaccessibility of β-carotene in stir-fried versus boiled sweet potato. Using an in vitro simulated digestion model, Failla et al. obtained a similar result, showing the influence of fat. Without fat, the bioaccessibility of the all-trans-β-carotene in the boiled orange-fleshed sweet potatoes ranged 0.6–3%. With the addition of 2% soybean oil, bioaccessibility increased more than two-fold, to 2.9–9.8%. In a different simulated in vitro digestion bioaccessibility study by Bechoff et al., the bioaccessibility of carotenoids in orange-fleshed sweet potatoes prepared by different processing methods was assessed. The Ugandan breads chapatis and mandazis were prepared using orange-fleshed sweet potato flour with oil and resembled large pancakes and square cakes, respectively. The bioaccessibility of carotenoids was substantially higher in chapatis and mandazis (73% and 49%, respectively) cooked with oil than in orange-fleshed sweet potato porridge and puree (16.3% and 9.9%, respectively) cooked without oil. This study supports evidence that oil added prior to or during cooking can effectively increase the potential for β-carotene bioavailability, while oil added after may not.

Those studies also compared the bioaccessibility of the different β-carotene isomers. All studies found better absorption of cis isomers than of all-trans isomers. cis isomers are believed to possess half the vitamin A biological activity of the all-trans form. In the study by Mills et al., cis isomer bioaccessibility was 10-fold higher than in orange-fleshed sweet potato porridge and puree. Those studies also compared the bioaccessibility of the different β-carotene isomers. All studies found better absorption of cis isomers than of all-trans isomers. cis isomers are believed to possess half the vitamin A biological activity of the all-trans form. In the study by Mills et al., cis isomer bioaccessibility was 10-fold higher.
Table 3 Provitamin A carotenoid bioavailability studies.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Meal or processing method</th>
<th>Model</th>
<th>All-trans bioavailability (% absorption)$^a$</th>
<th>Final all-trans-β-carotene content (μg/g FW)$^a$</th>
<th>Final cis-β-carotene content (μg/g FW)$^a$</th>
<th>cis-isomer bioavailability (% absorption)$^a$</th>
<th>Final cis-β-carotene content (μg/g FW)$^a$</th>
<th>No. of cultivars</th>
<th>Notes</th>
<th>Data type$^b$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>9.9</td>
<td>95</td>
<td>43.5 (13-cis)</td>
<td>7.4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Bechoff et al. (2011)$^{131}$</td>
</tr>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Porridge</td>
<td>Simulated digestion</td>
<td>16.3</td>
<td>8.7</td>
<td>30.3 (13-cis)</td>
<td>1.2</td>
<td>1</td>
<td>7.4% fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Mandazis</td>
<td>Simulated digestion</td>
<td>49.0</td>
<td>32.9</td>
<td>98.1 (13-cis)</td>
<td>3.7</td>
<td>1</td>
<td>3.3% fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Chapatis</td>
<td>Simulated digestion</td>
<td>72.7</td>
<td>31.5</td>
<td>96.2 (13-cis)</td>
<td>2.5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>0.6–3.0 (w/o fat)</td>
<td>99.6–265.1</td>
<td>5.3–24.5 (13-cis)</td>
<td>15.6–33.9</td>
<td>8</td>
<td></td>
<td></td>
<td>Data calculated</td>
<td>Failla et al. (2009)$^{152}$</td>
</tr>
<tr>
<td>Orange-fleshed sweet potato</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>2.9–9.8 (w/fat)</td>
<td>13.2–26.3 (13-cis)</td>
<td>16.3–33.9</td>
<td>2% soybean oil</td>
<td>Data calculated</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cassava</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>1.2 (w/o fat)</td>
<td>269.0</td>
<td>12.7 (13-cis)</td>
<td>11.2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Mills et al. (2009)$^{153}$</td>
</tr>
<tr>
<td>Cassava</td>
<td>Raw</td>
<td>Simulated digestion</td>
<td>2.8 (w/fat)</td>
<td>263.4</td>
<td>NA</td>
<td>16.8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Gomes et al. (2013)$^{150}$</td>
</tr>
<tr>
<td>Cassava</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>5.4</td>
<td>1.1</td>
<td>NA (total-cis)</td>
<td>0.5</td>
<td>1</td>
<td>0.6 g fat</td>
<td></td>
<td></td>
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<tr>
<td>Cassava</td>
<td>Fried</td>
<td>Simulated digestion</td>
<td>6</td>
<td>0.77</td>
<td>NA (total-cis)</td>
<td>0.76</td>
<td>1</td>
<td>0.5 g fat</td>
<td></td>
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<tr>
<td>Cassava</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>14.1</td>
<td>1.6</td>
<td>NA (total-cis)</td>
<td>1.43</td>
<td>1</td>
<td>11 g fat</td>
<td></td>
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<tr>
<td>Cassava</td>
<td>Gari</td>
<td>Simulated digestion</td>
<td>27.5</td>
<td>NA</td>
<td>26 (9-cis)</td>
<td>NA</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>Thakkar et al. (2009)$^{17}$</td>
</tr>
<tr>
<td>Cassava</td>
<td>Fufu</td>
<td>Simulated digestion</td>
<td>28.5</td>
<td>NA</td>
<td>26 (13-cis)</td>
<td>NA</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassava</td>
<td>Boiled</td>
<td>Simulated digestion</td>
<td>13.5</td>
<td>NA</td>
<td>16 (9-cis)</td>
<td>NA</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassava</td>
<td>Caco-2</td>
<td>Simulated digestion</td>
<td>30.0 (total β-carotene)</td>
<td>0–6.9</td>
<td>NA</td>
<td>NA</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>Thakkar et al. (2007)$^{159}$</td>
</tr>
<tr>
<td>Maize</td>
<td>Flour</td>
<td>Simulated digestion</td>
<td>16.7</td>
<td>0.7–9.0</td>
<td>9.1 (9-cis)</td>
<td>NA</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>Thakkar &amp; Failla (2008)$^{144}$</td>
</tr>
<tr>
<td>Maize</td>
<td>Caco-2</td>
<td>Simulated digestion</td>
<td>2.3</td>
<td>5.4 (13-cis)</td>
<td>NA</td>
<td>NA</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FW, fresh weight; NA, data not available; w, with; w/o, without.

$^a$ Ranges are given when different cultivars were tested.

$^b$ For some β-carotene concentration data and micellerization percentages, text did not give exact data and instead presented the data in bar graphs. In these cases, an estimate was made. When this was done, it was noted in the table. In other cases, the β-carotene concentration per gram was not explicitly stated. As a result, it was calculated and noted in the table.
than that of all-trans forms. Likewise, in the study by Bechoff et al.,131 cis isomers were more bioaccessible than the all-trans forms in all types of processed orange-fleshed sweet potato. Further interesting results were obtained in the study by Failla et al.132 Despite the 13-cis isomer representing only 7.1–14.3% of the total β-carotene in the orange-fleshed sweet potatoes, the amount of 13-cis absorbed (1.8–6.8 μg/g fresh weight [FW]) was slightly more than that of all-trans (1.4–5.1 μg/g FW). This equated to a bioaccessibility range of 5.3–24.5% for 13-cis. Interestingly, while the addition of 2% soybean oil resulted in a 2-fold increase in all-trans absorption, cis-isomer absorption remained nearly the same (2.4–8.3 μg/g FW; 13.2–26.3%). This is probably because cis isomers of β-carotene are naturally more water soluble than all-trans isomers and thus do not benefit as much from the inclusion of additional fat.

Despite the improvements in provitamin A bioavailability observed with food processing of biofortified sweet potatoes and other biofortified crops to be discussed, some processing techniques resulted in substantial carotenoid loss.17,125 Thus, improving bioavailability observed with food processing of biofortified sweet potatoes and other biofortified crops may be of lesser concern.

Cassava

Two similarly designed human studies reported a high β-carotene bioavailability and a very efficient bioconversion of biofortified cassava. Both studies measured changes in the triacylglycerol-rich lipoprotein fraction of healthy North American women who consumed a biofortified cassava porridge. Liu et al. (W. Liu, unpublished data, 2009) found a bioconversion rate of 2.8:1 in a low-fat meal. A second study by La Frano et al.119 measured bioconversion rates of 4.2:1 when cassava was consumed with a high-fat meal (containing 20 g of fat) and 4.5:1 when consumed with a moderate-fat meal (containing 6 g of fat).119 The moderate-fat meal contained an amount of fat considered to be sufficient for optimal carotenoid absorption, which most likely explains the lack of a significant difference between the two meals.

Most of the results from one animal study and several in vitro studies have shown high carotenoid absorption from biofortified cassava.17,137–139 A recent report, however, suggests that the bioavailability of carotenoids may be highly variable.138

The bioconversion efficiency of biofortified cassava has been studied in Mongolian gerbils. In a study of vitamin-A-deplete gerbils, the bioconversion efficiency of biofortified cassava was 3.7:1, despite high concentrations of cis isomers in the cassava.137 This was a surprising result, since cis isomers are believed to have far less bioconversion efficiency. It may suggest the bioconversion of cis isomers is more efficient than currently believed.

A study by Thakkar et al.17 investigated the impact of traditional West African food-processing techniques, including boiling and making gari and fufu, on the bioavailability of β-carotene from cassava-based foods. The cassava product gari is fermented, roasted, and granular in texture, while fufu is cassava in the form of a wet paste. The 9- and 13-cis isomers accounted for 30–45% of the total β-carotene in the cultivars tested. Bioaccessibility of the all-trans and cis isomers was similar. The efficiency of uptake into the micellar fraction for boiling and gari was relatively high (27.5% and 28.5%, respectively). The bioaccessibility from fufu, however, was only 13.5%. The reason for the substantial decline in bioaccessibility is unclear. To make interpretations even more difficult, a subsequent study by the same group testing transgenic biofortified cassava observed similar results between boiled cassava and fufu, but lower bioaccessibility from gari.140

Thakkar et al.139 investigated β-carotene micellization from biofortified cassava using a combination of simulated in vitro digestion and the Caco-2 cell model. For the 10 cultivars studied, bioaccessibility of total β-carotene as measured by simulated in vitro digestion was 30%. The amount of β-carotene partitioned into micelles and accumulated by Caco-2 cells was linearly proportional to β-carotene concentrations in the cultivars tested. As observed by Thakkar et al.,17 the efficiency of micellization appears to be unaffected by the concentration of β-carotene in the cultivar and appears to be independent of the cultivar. Since the ultimate goal is to raise concentrations as high as possible, it is promising to observe that concentration effect and cultivar selection may be of lesser concern.

A recent study indicates that bioavailability of β-carotene from biofortified cassava may vary substantially, even when similar cooking methods are used. Gomes et al.138 observed bioaccessibility to be 5.4%, 6.0%, or 14.1% for raw, boiled, and fried cassava, respectively. The reason for the relatively low bioaccessibility of β-carotene in the boiled cassava may have been due to the complex homogenization procedure performed during the in vitro digestion protocol. This procedure formed a starch gel that might not have fully interacted with the digestive fluids, thereby inhibiting the release of carotenoids from its matrix and reducing the efficiency of micellization. This possibility was supported by fluorescence microscopy of the sample that identified the presence of amorphous complexes capable of entrapping carotenoids and decreasing micellization.138

Maize

Two human studies have indicated that the provitamin A carotenoids in biofortified maize efficiently convert to vitamin A.

A study by Li et al. measured the triacylglycerol-rich lipoprotein fraction of human blood estimated biofortified maize porridge (containing 8 g of fat) to have a vitamin A equivalence of 6.5:1 in North American women. In a study conducted by Muzhingi et al. the vitamin A equivalence of intrinsically labeled high-β-carotene yellow maize porridge (containing 21 g of fat) was estimated as 3.2:1 in Zimbabwean men. Both of these studies indicate that consumption of biofortified maize may supply sufficient vitamin A to meet daily requirements.

An animal study testing bioavailability and an in vitro simulated digestion/Caco-2 cell study also testing bioaccessibility of biofortified maize have supported the findings of human studies in terms of efficient absorption. In addition, neither study observed a negative influence of the high concentration of the large variety of carotenoids in maize on absorption.

A Mongolian gerbil feeding study indicated that biofortified maize containing high concentrations of β-cryptoxanthin resulted in a more efficient bioconversion (2.4:1) than did a β-carotene supplement (4.6:1). It is interesting to note that the biofortified maize contained relatively high concentrations of the provitamin A β-cryptoxanthin. This research indicates that biofortified maize containing both β-carotene and β-cryptoxanthin can effectively increase vitamin A stores.

In an in vitro simulated digestion/Caco-2 cell model study by Thakkar and Failla, the micellerization efficiency of β-carotene in biofortified maize was found to be 16.7%/14.1%. Biofortified maize is known to contain a larger variety of carotenoids than other biofortified foods. In fact, the maize contained a large number of xanthophylls, such as lutein and zeaxanthin, that have been suspected to negatively affect β-carotene absorption. However, similar to the previously mentioned gerbil study, results indicated that the presence of other carotenoids in maize does not negatively impact bioavailability. It is suspected that the results may differ from those of previous studies performed using carotenoid supplements because the study was performed in whole food. In the Caco-2 cell model, β-carotene absorption decreased in a concentration-dependent manner as lutein concentrations increased. The authors believed this was likely due to competitive inhibition for the SR-B1 receptor rather than to lutein directly.

In summary, human studies provide the most reliable indication of the bioavailability of micronutrients in biofortified crops, whereas animal and in vitro studies are useful for within-study comparisons of absorption factors and screening of the varieties developed through breeding. The results of the human bioavailability studies for iron (2.6–9.0%) and zinc (17–20%) in biofortified crops were consistent with the results of studies in nonbiofortified plants. In contrast, the bioconversion of β-carotene to retinol in the provitamin-A-biofortified crops, which varied from 2.8:1 to 6.5:1, was more efficient than the average bioconversion of 12:1 estimated for nonbiofortified foods by the Institute of Medicine.

Concerns about the bioavailability of micronutrients from crops are much different for minerals than for carotenoids. The bioavailability of provitamin A carotenoids is most affected by dietary habits and food processing. The addition of fat to a meal improves absorption, while extreme food processing, such as treatment with high heat, can result in substantial carotenoid loss. The bioavailability of iron and zinc, on the other hand, is influenced primarily by the presence of antinutrients such as phytate. Although phytate levels can be reduced through plant breeding, decreasing them too much may diminish or eliminate the benefits that phytates provide to plants and, potentially, to humans. Thus, to minimize the negative effects of food processing and the factors that adversely influence bioavailability, biofortification to increase micronutrient concentrations in staple crops remains the primary goal.

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135. Deming DM, Baker DH, Erdman JW. The relative vitamin A value of 9-cis β-carotene is less and that of 13-cis β-carotene may be greater than the accepted 50% that of all-trans β-carotene in gerbils. J Nutr. 2002;132:2709–2712.


