Article to the Special Issue

Provitamin A Carotenoid Bioavailability: What Really Matters?

Sherry A. Tanumihardjo [1], Natalia Palacios [2] and Kevin V. Pixley [2,3]

[1]Department of Nutritional Sciences, University of Wisconsin-Madison, WI, USA; [2]International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico; [3]Department of Agronomy University of Wisconsin, Madison, WI, USA

Abstract: Micronutrient malnutrition, or “hidden hunger,” afflicts a large part of the world’s population, with vitamin A deficiency among the most prevalent public health problems. Provitamin A carotenoids in plant foods are a source of vitamin A for humans; however, several factors, including species of carotenoids, host status, and effectors of absorption can negatively, positively, or in yet undetermined ways affect the bioavailability of these compounds. Staple foods biofortified with provitamin A carotenoids have shown more efficient bioconversion to retinol than generally observed for vegetables (e.g., 3–6 versus 10–80 μg β-carotene to 1 μg retinol). Staple foods such as maize, rice, and cassava, are generally more accessible than meat or vegetable sources of retinol or provitamin A carotenoids to poor consumers, who are most likely to suffer micronutrient malnutrition. Interdisciplinary teamwork, including plant breeders, nutritionists, government and local agencies, seed companies, and communities, is needed to avail biofortified crops to needy populations. Key steps include developing, validating the nutritional effects of, providing nutrition education concerning, and promoting the use of biofortified crops. Provitamin A carotenoid biofortification of sweet potato, maize, cassava, and rice are at different stages along this continuum. Close linkages between agriculture, nutrition, and health, are essential in the quest to eradicate hunger among the poor.

Key words: provitamin A carotenoids, bioavailability, maize, rice, cassava, biofortification

Introduction

Carotenoids are a group of more than 700 phytochemicals with a broad range of structures and polarities. About 50 carotenoids can be cleaved to vitamin A, but only three represent major sources in the human diet; i.e. β-carotene, α-carotene, and β-cryptoxanthin (Figure 1). Even though sources of provitamin A carotenoids are plentiful in the world, vitamin A deficiency (VAD) affects over 250 million people globally and is one of the most prevalent nutritional deficiencies in developing countries, resulting in growth, vision, reproduction, and immunity impairments [1]. Provitamin A bioavailability is an issue in meeting vitamin A needs in populations that rely heavily on plant-based diets. Bioavailability of pre-formed retinol is not limited and most dietary retinol is absorbed and utilized, distributed to essential organs, or stored in the liver [2]. The liver has a large capacity to store vitamin A as retinyl ester for either later use or as a long-term depot to mitigate toxic effects that would occur when dietary intake is high [3]. Animal-based foods that are high in pre-formed vitamin A; e.g., liver, eggs, and fortified milk, are often not available or are expensive to consumers among the poor and food-insecure [4].

Carotenoid bioavailability continues to be an active research interest, especially considering the agricultural push to biofortify staple crops with provitamin A carotenoids [5, 6]. In 1996, several factors were proposed that may affect carotenoid bioavailability and the term SLAMENGHI [7] was formulated as a mnemonic: Species of carotenoid, molecular Linkage, Amount of carotenoids in a meal, Matrix in which the carotenoid is incorporated, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors, and mathematical Interactions. These factors were further reviewed by Castenmiller and West in 1998 [8]. Much research has been performed revolving around many of these factors in the past decade in regard to provitamin A carotenoids, but questions still remain with regard to specific foods, nutrient interactions, and the amounts needed to improve vitamin A status. Recently, a series of studies with plant foods used sensitive stable isotope methodology to measure changes in liver vitamin A reserves in response to intervention [5, 9]. These

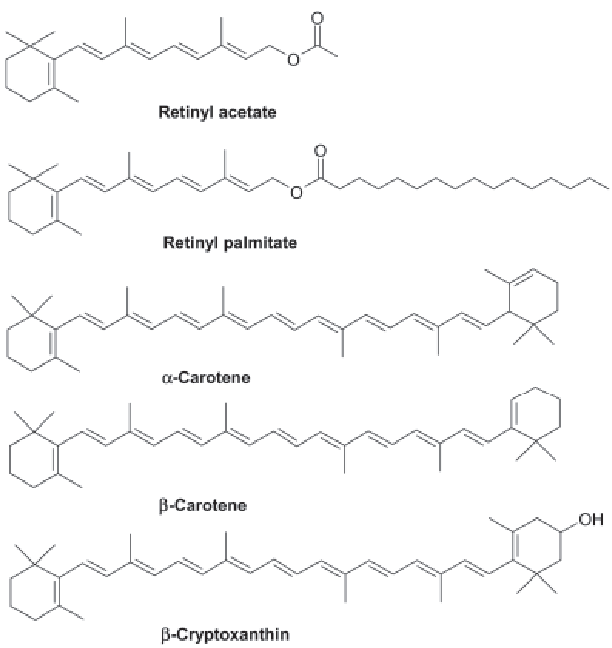

Figure 1: The different forms of vitamin A found in supplements, fortified foods, and plant foods. The pre-formed vitamin A as acetate and palmitate esters can be found in oil-based or water-miscible forms. The water-miscible forms tend to be more toxic. The provitamin A carotenoids are found in fruits and vegetables, e.g., α-carotene in carrots, β-carotene in spinach, and β-cryptoxanthin in citrus fruits and maize.

studies followed a study in Indonesian women, which found a lack of improvement in vitamin A status using less sensitive methodologies [10].

The basic understanding of the digestion and metabolism of carotenoids [11] is needed to interpret new research on host and effector influences on carotenoid bioavailability. In review, the process of mastication and exposure to stomach enzymes and acid, dissolves the carotenoids into lipid droplets. In the small intestine, carotenoids are incorporated into mixed micelles, which demonstrate a finite capacity for carotenoid incorporation [12]. This phenomenon may explain poor uptake when carotenoid concentrations are high. Carotenoids move from the duodenum into the mucosal cells of the intestine by passive diffusion. The provitamin A carotenoids can be cleaved to retinal and reduced to retinol (Figure 2) or incorporated directly into chylomicra with the non-provitamin A carotenoids for circulation through the lymphatic system into the bloodstream.

The process of converting provitamin A carotenoids to retinol for utilization by the body is complex [13]. Bioaccessibility is the amount of β-carotene that is released from the food matrix and available for absorption. Bioavailability is defined as the fraction of carotenoid that is absorbed and available for utilization in normal physiological functions or for storage. Bioconversion is the proportion of absorbed provitamin A carotenoid converted to retinol. A bioconversion rate of 100 % means that all of the absorbed β-carotene is converted to retinal and reduced to retinol. Bioefficacy combines absorption and bioconversion and is defined as the efficiency with which ingested dietary provitamin A carotenoids are absorbed and converted to active retinol [14]. A bioefficacy of 100 % means that 1 μmol dietary β-carotene results in 2 μmol retinol. The relationship of these terms to each other is depicted in Figure 2.

Bioconversion factors in the literature range from 2 μg for β-carotene dissolved in oil to greater than 76 μg provitamin A carotenoids in green leafy vegetables equal to 1 retinol equivalent (RE = 1 μg retinol) [15]. The traditional retinol conversion factors from the National Research Council (1989) of 6 μg β-carotene and 12 μg for other provitamin A carotenoids to 1 μg retinol [16] were changed by the Institute of Medicine to reflect the research findings from several human feeding studies. They increased the retinol equivalency factor to 12 μg β-carotene and 24 μg for other provitamin A carotenoids; i.e. β-cryptoxanthin and α-carotene, to 1 μg retinol activity equivalent (RAE) [17]. Because of this difference among the bioconversion factors and carotenoids, food composition data

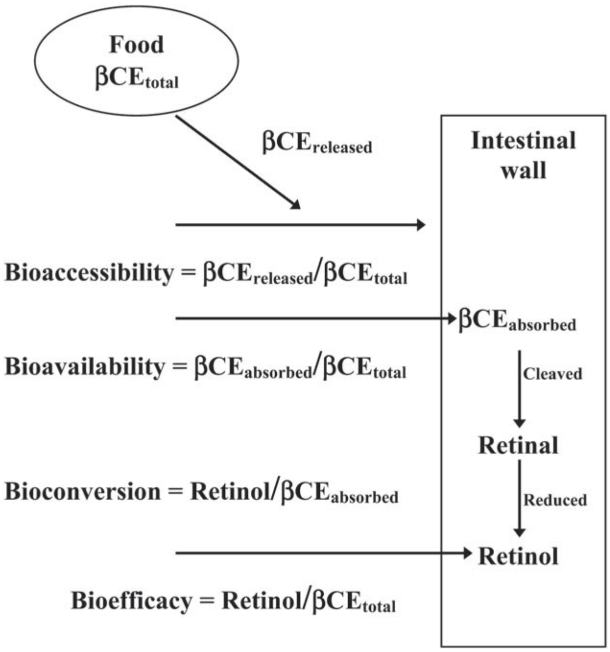

Figure 2: The various terms that relate to provitamin A bioavailability (adapted from [13]). βCE refers to β-carotene equivalents and is usually equal to β-carotene plus ½ α-carotene and ½ β-cryptoxanthin.

tables should report food content in the amount of each carotenoid whenever possible [17]. The Food and Agricultural Organization continues to use the 6 μg β-carotene to 1 μg retinol conversion factor [18], and this conversion factor was recently obtained for maize with enhanced levels of β-carotene [19]. Therefore, reading prior and future research requires cognizance of the carotenoid profile, bioconversion factor used, and their bearing on conclusions drawn from the study.

The process of biofortification, intrinsically enhancing nutrients and/or nutrient bioavailability in crops either through traditional breeding methods or transgenic approaches [6, 20, 21], has spurred new resources for investigation of provitamin A bioavailability from staple crops. Development of a biofortified variety requires time and involves several steps, including: 1) wide-scale screening to identify or develop lines with a high concentration of the desired nutrient for breeding biofortified cultivars, 2) crossing elite lines with these sources, 3) selecting new cultivar types combining high levels of the nutrient with excellent yield and other agronomic traits, 4) extensive testing and validation of performance, 5) validation of the suitability for use in food products, and 6) registration and commercialization of the new variety.

A broad range of disciplines are needed to support biofortification from field to table (Table 1). This agricultural-based approach to increase the nutrient density of staple crops is a promising strategy for improving the nutrition, health, and livelihoods of populations, especially of the rural poor who consume predominantly staple crops [22, 23]. This review will discuss recent advances in provitamin A carotenoid bioavailability and biofortification efforts.

Table I: Some of the disciplines involved in establishing a biofortified crop from seeds to bowls

Disciplines	Tasks
Biology (plant biochemistry, molecular biology)	Knowledge and cutting-edge technologies for rapid breeding of biofortified varieties
Plant breeding and agronomy	Development and testing of the agricultural suitability of biofortified varieties
Plant physiology	Knowledge of metabolic pathways to enhance efficiency of breeding and foresight of selection effects (biofortification of seed) on plant function
Analytical chemistry	Method development to analyze nutrients in crops, screen crops for nutrient concentrations
Food technology	Food product development, optional markets, retention studies
Nutrition	Test food products in animal and human models, nutrition education
Socio-economics	Market, impact, acceptability
Extension	Farmer-participatory variety evaluation, validation and demand-creation
Education	Behavior change
Medicine	Morbidity studies, health status of target populations
Communication	Product dissemination, nutrition education, public and private sector awareness and engagement
Seed technology	Maintenance and production of high-quality, affordable seed parent stocks and commercial seed

Recent Advances in Provitamin A Carotenoid Bioavailability

A three-step process for nutritional studies is recommended during the advanced stages of developing biofortified varieties: 1) tests for bioavailability using *in vitro* and animal models; 2) short-term, highly controlled feeding trials to test efficacy in target populations; and 3) longer-term effectiveness trials evaluating the nutritional, health, agricultural, and environmental impact of the novel foods on the community [21]. This research continuum is needed to accomplish the goal of getting provitamin A carotenoid-biofortified crops on the table. Along this continuum, several factors affecting bioavailability have been identified as important for consideration and further research. These include: <u>S</u>pecies of carotenoid, <u>H</u>ost-related factors, <u>E</u>ffectors of absorption, <u>R</u>elative amounts of carotenoids, <u>R</u>esistant starch, and <u>Y</u>et to be determined. Thus, a simpler mnemonic is coined, the SHERRY factors. These factors will be defined in light of recent research to support provitamin A biofortification efforts.

Species of carotenoid

The species of provitamin A carotenoid, whether it is a hydrocarbon or hydroxy-containing, affects the bioavailability. In Mongolian gerbils, α- and β-carotene bioefficacies were directly compared [24]. As predicted due to similar polarities, twice the amount of purified α-carotene maintained vitamin A status as well as β-carotene in gerbils on a vitamin A-depletion regimen. Bioconversion factors were ~5.5 μg α-carotene and ~2.8 μg β-carotene to 1 μg retinol [24], which are slightly higher than the Institute of Medicine's values of 4:1 and 2:1 for these provitamin A carotenoids in oil, respectively [17]. On the other hand, in an identically designed study comparing β-cryptoxanthin with β-carotene dissolved in oil, very similar bioconversion factors, i. e., 2.74 and 2.52 μg to 1 μg retinol, respectively, were obtained [25], suggesting more effective micellerization and absorption of β-cryptoxanthin. β-Cryptoxanthin and β-carotene are the major provitamin A precursors in maize. When β-cryptoxanthin-biofortified maize was fed to Mongolian gerbils in a separate experiment, the bioconversion factor was 2.4 μg β-carotene equivalents to 1 μg retinol and that of an equivalent β-carotene supplement was 4.6 μg β-carotene to 1 μg retinol [25]. The bioconversion factors (i. e. 2.1–3.3 μg β-carotene equivalents) obtained with biofortified maize with varying ratios of β-cryptoxanthin to β-carotene [26] were similar to that obtained with β-carotene-biofortified maize, which was 2.8 μg β-carotene to 1 μg retinol [27].

Efficient bioconversion factors (i. e., 6.48 ± 3.51 μg β-carotene to 1 μg retinol) were observed in well-nourished women fed biofortified maize (526.8 μg β-carotene equivalents) compared with a vitamin A reference dose (285.6 μg) using a chylomicron response test [19]. Furthermore, bioconversion was 3.0 ± 1.5:1 using stable isotope methodology in healthy Zimbabwean men fed biofortified maize [28]. These human studies show promise for biofortified maize impacting vitamin A status.

Typical yellow and biofortified maize contain high levels of the dihydroxy carotenoids lutein and zeaxanthin. Both *in vitro* work [29] and studies in Mongolian gerbils [26] found that the xanthophyll profile did not influence provitamin A bioefficacy. These studies suggest that bioefficacy of provitamin A carotenoids may not be compromised with high levels of other carotenoids, allowing biofortification strategies to focus both on increasing total carotenoid as well as provitamin A carotenoid content, specifically.

Another species effect is whether or not the *cis/trans* configuration influences bioefficacy. In studies conducted in animals with yellow biofortified cassava, the bioconversion factor was 3.7 μg β-carotene to 1 μg retinol, despite 48 % *cis*-β-carotene composition [30]. Supplement studies comparing the isomers revealed that all-*trans*-β-carotene is more efficacious than either 9-*cis*- or 13-*cis*-β-carotene [31]. When 9-*cis* was compared with 13-*cis*, one study showed that 9-*cis* was less bioefficacious [32], but another study did not show a difference between the two *cis*-isomers [33]. Thus, the effect of *cis/trans* configuration as it relates to provitamin A biofortification efforts has not been completely resolved.

Host-related factors

Single nucleotide polymorphisms in the human *BCMO1* gene have been discovered causing observably reduced 15, 15'-β-carotene monooxygenase 1 activity, which centrally cleaves provitamin A carotenoids to vitamin A [34]. Individuals with this polymorphism cleave provitamin A carotenoids to retinol at a reduced level. Although this is an important consideration at the individual level, the influence on biofortification efforts at the population level is unknown.

One of the driving forces for provitamin A bioefficacy is the vitamin A status of the host [5]. By combining data from multiple, similarly designed studies testing various foods in Mongolian gerbils, it is possible to conclude that as liver reserves decreased, bioconversion increased (Figure 3). In Filipino school children, bioconversion of plant provitamin A carotenoids to vitamin A also varied inversely with vitamin A status [35]. Therefore, as biofortification efforts continue, the amount of provitamin A can continue to be increased without the concern of toxicity, as can occur with fortification of foods with pre-formed vitamin A [5, 36].

Nutrients often work together in the body and one may be necessary for the other's absorption, metabolism, or transport. Thus, food-based approaches and biofortification of staple crops are attractive strategies to improve nutrition by simultaneously providing more than one nutrient. The link between vitamin A status and iron deficiency anemia continues to be studied and improvement in iron status is repeatedly seen when vitamin A and iron are given together [37–40]. Vitamin A is involved in the formation of red blood cells, modulation of the anemia of infection, iron mobilization and transport, and modulation of immunity through lymphocyte production and adhesion [41].

Furthermore, zinc (Zn) and vitamin A (Figure 4) are required together in the body; therefore, poor Zn status will negatively impact vitamin A status. Zn is a probable co-factor for the action of the 15, 15'-β-carotene monooxygenase [42] and Zn deficiency depresses the hepatic synthesis of the carrier protein of vitamin A resulting in lower plasma retinol concentrations [43]. Zn is also required in the action of retinol dehydrogenase to oxidize retinol to retinal, the essential pigment for vision [43, 44]. If a staple crop, such as maize, was biofortified with both provitamin A carotenoids and Zn, the improvement in Zn status would further enhance vitamin A metabolism and perhaps function. A similar situation would be expected if provitamin A-biofortified maize is part of a Zn-adequate diet.

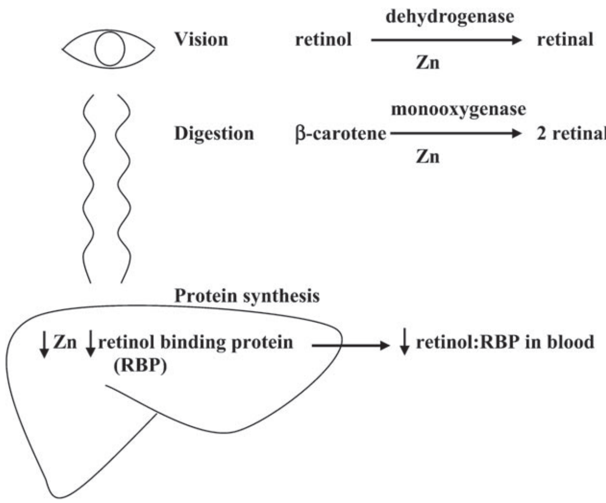

Figure 4: Biological functions where vitamin A (retinol) and zinc (Zn) work together in the human body. Zn is an important co-factor in enzymes and protein synthesis.

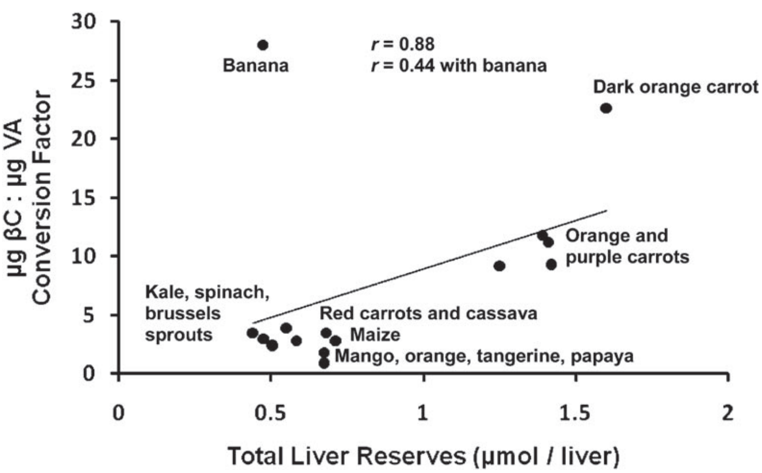

Figure 3: The relationship of the bioconversion factor for β-carotene equivalents (βC) to vitamin A (VA) as it relates to total liver reserves in Mongolian gerbils fed multiple fruits and vegetables. Provitamin A carotenoid from banana is a notable outlier. (Adapted from [5] by Arscott SA).

Effectors of absorption

Fat is known to be essential for the absorption of carotenoids. However, the amount of fat needed is minimal. In schoolchildren fed 4.2 mg provitamin A carotenoids for 9 weeks as carrots, bok choy, squash, and kangkong, with 2.4, 5, or 10 g fat/meal, the amount of fat did not influence the amount of vitamin A produced [45]. In the same study, the prevalence of low retinol reserves in the liver (i. e., < 0.07 μmol retinol/g liver), determined by isotope dilution after the intervention, decreased from 35 % to 7 %. In Mongolian gerbils, bioconversion of provitamin A carotenoids from sweet potato was improved as the amount of fat in the diet increased from 3 to 12 % [46]. Emerging data from *in vitro* models suggest that dietary fats with an increased ratio of unsaturated to saturated fatty acids enhance the absorption of carotenoids by increasing both the efficiency of micellarization and lipoprotein secretion [47]. Application of these data to animal and human studies should follow in relationship to biofortified crops.

Relative amounts of carotenoids

Bioconversion is affected by the amount of provitamin A carotenoid fed, although thresholds have not been defined. After high-carotene biofortified carrots were fed to gerbils, liver vitamin A concentrations increased by 10 % over purple and typical orange carrots, while β-carotene concentration was doubled in the high-carotene group [48]. Varying the amounts of two varieties of biofortified cassava in the diet of gerbils resulted in a muted vitamin A response but a tripling of the β-carotene liver concentration [30]. Liver retinol reserves of gerbils increased with increasing β-carotene amounts from maize, and this is likely explained by low liver reserves favoring bioconversion to retinol [27]. The β-carotene liver concentration was doubled with maize feeding versus β-carotene supplementation and likely reflects the day-long exposure to β-carotene through *ad libitum* access to maize. After these favorable studies in gerbils and data available from human studies, the impact of biofortified maize on liver reserves of vitamin A was modeled [5]. Modeling reflected the influence of vitamin A status, provitamin A concentration, and bioconversion regulation on the impact that biofortified maize can have on retinol liver reserves in comparison to supplementation and fortification. In addition to a low vitamin A status favoring bioconversion, the amount of carotenoid fed will influence whether the provitamin A carotenoid is cleaved to retinol or absorbed, circulated intact, and stored in body tissues.

Resistant starch

Staple crops that are targeted for provitamin A carotenoid biofortification have different carbohydrate and fiber compositions (Table 2) [49]. We hypothesize that resistant starch, which is starch that can escape digestion and act as a dietary fiber in humans, may be negatively impacting provitamin A carotenoid bioaccessibility and needs to be carefully evaluated. Unfortunately, resistant starch is not routinely measured in foods and changes during processing and cooking.

Efficient bioconversion factors have been measured in human studies with maize [19, 28], cassava [50], and Golden Rice [51, 52]. Human studies with sweet potato, however, have shown higher bioconversion factors; e. g., 13.4 μg β-carotene to 1 μg retinol, measured using stable isotope methodology, when sweet potato and pre-formed vitamin A were fed to different groups of Bangladeshi men [53]. In South African schoolchildren, liver vitamin A reserves improved after 5 months of feeding a single serving of orange-fleshed sweet potato in the morning before lunch [54]. A modeling study predicted that an infant eating 100 g sweet potato/day would have gradual improvement in liver reserves [5]. Increasing the percentage of sweet potato through addition of 3 and 9 % white-fleshed sweet potato powder to a 3 % orange-fleshed sweet potato powder diet (i. e. 3, 6, and 12 % total sweet potato powder), did not influence the bioconversion factor in gerbils [46], which is important from a breeding standpoint because many consumers prefer sweet potatoes with high dry matter content.

An effectiveness study promoted orange-fleshed sweet potato in Mozambique using a two-year integrated agricultural and nutrition intervention covering two agricultural cycles [55]. Ninety percent of the intervention households produced orange-fleshed sweet potato and the intervention children ate orange-fleshed sweet potato more often than control children. Serum retinol concentrations were higher in the intervention than the control children, demonstrating efficacy [55]. The intervention included taste evaluations and marketing of sweet potato flour. The addition of sweet potato flour to wheat flour was used by local bread makers, in part to reduce costs, and the product called "Golden Bread" [56]. The success of sweet potato in Africa required coordination among many disciplines, local organiza-

Table 2: The carbohydrate and fiber composition of selected staple crops obtained from reference [49]. Resistant starch, which is starch that may act like fiber in the body, is not routinely measured due in part to changes during processing and cooking.

Food (100 g)	Carbohydrate	Fiber	Water	% Carbohydrate of dry matter[1]	% Fiber of dry matter[1]
Rice, dry	80.0	1.3	11.6	90.5	1.47
Corn meal, dry	76.9	7.3	10.3	85.7	8.14
Sweet potato, raw	20.1	3.0	77.3	88.5	13.2
Banana, raw	22.8	2.6	74.9	90.8	10.4

[1] Combined values > 100% based on dry matter likely reflect differences between studies used by the USDA to compile the database [49].

tions, stakeholders, and government ministries. Release of orange sweet potato varieties that were agronomically equal to white was crucial. Farmers participated in varietal selection, seed systems were developed, and the product successfully marketed. Promoting the nutritional and economic values was synchronized with the release of adapted varieties to farmers.

Progress with dissemination of Golden Rice has been slower than sweetpotato and biofortified maize. The highest carotenoid concentration recorded to date for Golden Rice is 37 μg/g dry weight [57], and rice with values of 5 and 20 μg/g have been fed to humans [51]. In 5 healthy adults, the conversion factor for Golden Rice β-carotene to retinol was 3.8 ± 1.7 μg to 1 μg with a range of 1.9–6.4 to 1 [51]. When fed to Chinese children (n = 24), pure β-carotene, Golden Rice β-carotene, and spinach β-carotene to retinol bioconversion factors were 2.0, 2.1, and 7.3 μg to 1 μg, respectively [52]. These efficient bioconversion factors likely reflect the low level of β-carotene in the rice as well as good bioaccessibility from the highly digestible starch matrix [51]. Therefore, a significant impact on vitamin A status could be achieved if it was adopted by the groups that need it. Currently, high β-carotene rice varieties adapted to the United States growing environment are being back-crossed into popular high-yielding varieties which grow well in Asia and will soon be field-tested at the International Rice Research Institute in the Philippines.

Preliminary research with carotenoid-containing bananas on vitamin A status compared with other fruits was assessed in gerbils [58]. The bioconversion factor obtained from banana was not as favorable as for the other fruits (Figure 3) and may be related to their provitamin A profiles. Banana varieties contain varying levels of carotenoids and the identification of carotenoid-rich cultivars, targeting those areas of the world where bananas are a major staple food, is ongoing [59]. Further studies with banana are underway in animal models and preliminary results have also revealed poor bioavailability [60]. Matrix effects from banana and other staple crops should include evaluation of resistant starch and fiber type to further define their impact on bioavailability [46].

Yet to be determined

Whole-food approaches for nutrition are not new concepts, but are often overlooked and perceived as being expensive and not effective for specific nutrients such as vitamin A due to issues associated with provitamin A bioavailability. The whole-food matrix is important, however, because it interacts with the human body in beneficial ways that single nutrient approaches, such as supplementation, cannot duplicate. Although original work suggested that green vegetables are not a promising source of vitamin A in humans [10], more recent work in animals has shown efficient provitamin A bioefficacy from spinach, kale, broccoli, and African indigenous green leaves [58, 61]. Green vegetables can make a difference in vitamin A status, and horticultural development could help ensure their availability and consumption in appropriate amounts to make a difference in vitamin A status [62]. The vitamin A pool size of Chinese school-aged children was measured before and after a food-based intervention using either green and yellow vegetables or light-colored vegetables [63]. Total body vitamin A pools decreased in those children who were fed light-colored vegetables but remained constant in children who were fed green and yellow vegetables. The calculated equivalence was 26.7 μg β-carotene:1 μg retinol (range 19:1 to 48:1). Although this conversion factor is high, nutrition education to promote consumption of more vegetables should still be a major public health message for vitamin A intake and optimal nutrition.

Although high-dose vitamin A capsules given every six months to preschool children boost liver stores, the day-to-day intake of a biofortified food or a diet high in provitamin A-rich fruits and vegetables would not only meet daily vitamin A needs, but also provide other nutrients and phytochemicals leading to improved intestinal health. The gut is a major immune organ and the gut-associated lymphoid tissue (GALT) is maintained through interaction with the environment [64], which includes a healthy diet. This whole-food with gut interaction should be further explored with provitamin A biofortified crops in comparison with vitamin A supplements.

For many years, vitamin A supplementation with pre-formed retinyl esters (Figure 1) has continued in developing countries while food-based approaches have been downplayed [65]. The benefits of vitamin A supplementation are clearly described in the literature [66]; however, supplementation programs are relatively expensive and have recurring costs, especially considering the human resources to distribute the supplements [67]. Whole-food sources of provitamin A carotenoids have benefits in addition to improving vitamin A status; for example, when carrots were fed to animals, tissue antioxidant concentrations improved, compared with administering vitamin A supplements [68]. Studies with provitamin A carotenoid-biofortified crops should be done to define optimal health effects in addition to measuring vitamin A status.

Bioavailability: What really matters?

Healthy diets that include plant-based sources of provitamin A carotenoids; e. g., red palm oil, green vegetables, or biofortified staple crops, are the desirable approach to meet nutritional needs. While many things in life need to be taken in moderation, vegetables need to be taken in large quantities for optimal health. Current *MyPyramid* guidelines (3.5–4.5 cups vegetables and fruits as part of a healthy 1600–2000 kcal diet) [69] may induce weight loss or maintain weight in obese individuals if followed consistently [70]. Nutrition education messages to consumers encourage behavior change to improve diets, teach safe food storage techniques, and demonstrate preparation skills. Intense education efforts are essential to ensure a broad and lasting impact of nutrition and health interventions. Messages, while remaining holistic, need to explain and support biofortification and other complementary interventions aimed at improving and maintaining optimal health, especially for populations with chronic, limited access to well-balanced diets.

Recent Advances in Provitamin A Carotenoid Biofortification Efforts

Biofortification of staple crops is being adopted by the agricultural community with growing support by the nutrition and health communities [22, 23, 71]. Solving complex problems, such as poverty, food insecurity, and hunger, requires comprehensive approaches [4] and unique partnerships spanning from natural to social sciences, from researchers to politicians, and from developed to developing communities. Plant breeding and nutrition are just two of the critical fields for success of a biofortified crop approach to alleviate malnutrition. Other notable examples include social science to study consumer preferences, food technology to evaluate uses of the biofortified grain, agricultural extension to survey farmers and familiarize them with new varieties, marketing to develop effective strategies to promote the product, and more (Table 1). Sharing each other's fields by visiting and participating in even a small part of the work can improve understanding of the partnerships.

Benefits of an agriculturally-based approach to improve nutrition

Biofortification supports the first Millennium Development Goal (MDG) set forth by the United Nations Millennium Declaration in 2000 (Table 3), which is to "Eradicate extreme poverty and hunger", and an initial target is to reduce the proportion of people who suffer from hunger by 50 % [72]. Although several regions of the world have made progress, the recent global food and financial crises have created shortfalls in the required progress towards universally reaching this first goal [73]. Although foods consumed often meet caloric needs, "hidden hunger" can occur when nutritional requirements are not met and individuals suffer from subclinical nutrient deficiencies (e. g., iron, folic acid, and vitamin A), often without overt clinical signs of undernutrition [4].

Biofortification of staple crops with provitamin A carotenoids is a promising approach to end the hidden hunger of vitamin A in children and women at risk of deficiency. Undernutrition due to lack of food or poor food quality is widely recognized as a major contributor to childhood morbidity and mortality, highlighting the need for an expanded view of food security that includes nutrition, health, and quality of life. Children in rural areas are approximately twice as likely to be underweight than those in urban areas, particularly among the poor [73]. By improving important agronomic and nutritional traits of crops, rural populations are empowered to support themselves and improve the health of their children. Productive, biofortified crops offer a sustainable approach to improve nutrition in rural areas where infrastructure or resources to deliver supplements or fortified processed foods are lacking.

Agricultural development is intricately linked to poverty reduction [74] because increased agricultural productivity leads to income-generating opportunities through freeing time for greater participation in the labor market or marketing of crops [75] (Figure 5). Reduced poverty translates to enhanced food security, nutrition, and health, which is an underlying goal and essential input for many of the MDGs [4]. Agricultural development through nutritional enhancement (Table 3), which includes biofortification efforts, is an essential component of strategies to sustainably solve hidden hunger [76]. Successful biofortified crops will increase or maintain yields without increasing risk of crop (e. g., pest or disease attack) or income failure (e. g., market preferences).

Recent progress with provitamin A-biofortified maize along the research continuum

Maize varieties that combine high grain yield, good agronomic performance, and increased levels of

Table 3: Millennium Development Goals set forth by the United Nations Millennium Declaration in 2000 and their relationship to nutritional and agricultural interventions.

Goal	Description	Nutritional intervention	Agricultural input and/or outcome
1	Eradicate extreme poverty and hunger	Reduce undernutrition	Improve food availability Sustain the natural resource base Use of household income from crop production for food and health services
2	Achieve universal primary education	Improve cognitive development Improve health status	Enhance micronutrients Less demand for children to participate in crop production Fewer school absences
3	Promote gender equality and empower women	Reduce discrimination among family members during meals Nutrition education- behavior change	Empower women farmers Reduce health risks related to agricultural tasks
4	Reduce child mortality	Eradicate undernutrition in low income populations	Improve food systems to reach undernourished populations
5	Improve maternal health	Target interventions to improve micronutrient status	Better performance in the field
6	Combat HIV/AIDS, malaria and other diseases	Improve nutrition to prevent mortality	Healthier families to improve agricultural outputs
7	Ensure environmental sustainability	More effective use of available food	Improve cropping systems for sustainability
8	Develop a global partnership for development	Address hunger and malnutrition in multidisciplinary teams	Address food availability and improved crops in multidisciplinary teams

provitamin A carotenoids could enhance production while improving nutrition, health, and quality of life [23, 76]. Maize is the most important staple cereal crop in Sub-Saharan Africa, where consumption levels are high in some countries [77]. Maize is mainly a source of energy, providing over 20 % of total calories in human diets in more than 30 countries. As with most staple crops, over-dependence on maize results in poor health, stunted growth, reduced capacity for physical activity, and micronutrient deficiency.

Realizing the potential benefits of biofortified maize requires excellent science, multi-stakeholder action, and depends on demand from maize growers and consumers. Developing a global biofortification agenda requires ongoing discussion between plant scientists and nutritionists to understand the overlaps between these complex sciences. Crucial issues for plant breeding, such as limits to genetic variation and physiological function of nutrients in plants, are generally not known by nutritionists, while those related to

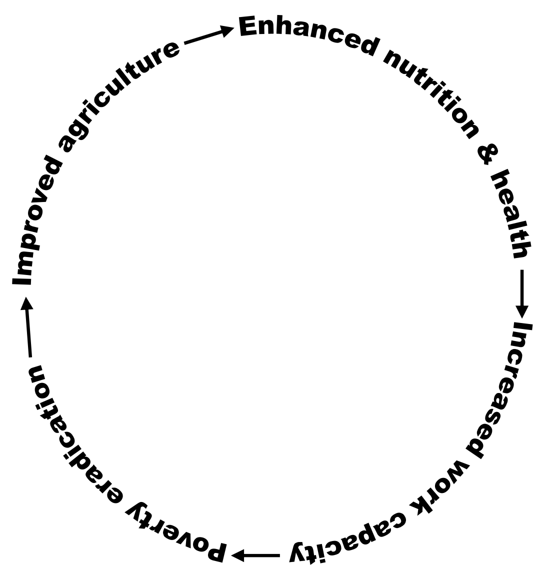

Figure 5: The interrelationship of agriculture and nutrition. One improvement will lead to and support the other in the quest to eradicate poverty and hunger. Improved agriculture includes biofortification of staple crops with micronutrients.

nutrition, such as nutrient bioavailability and retention, are unfamiliar to plant scientists. Nutritionists decided the target level of provitamin A carotenoids needed to show improvement in vitamin A status of biofortified maize consumers, which is currently set at 15 μg β-carotene equivalents/g dry weight [78], and breeders determined the feasibility and methods for achieving them. Setting and achieving coordinated short- and medium-term agendas among the disciplines is essential for the long-term goal to develop provitamin A-biofortified maize that can make a nutritional impact.

Studies have been completed with *in vitro* methods to determine bioaccessibility [79, 80] and animal models to demonstrate bioefficacy of provitamin A from biofortified maize varieties [25–27]. After investigating retention in a typical African recipe [81], human studies were performed to verify the animal-predicted bioconversion factors of provitamin A carotenoid to retinol [19, 28]. Recently, the field component of a large efficacy trial that required coordination among plant breeders, nutritionists, seed companies, local and national health agencies and ministries, biochemists, and entire communities was completed in Zambia. Maize that was grown in Zambia was fed to children for 70 days; however, while the children quickly adapted to the orange maize [82] and serum retinol concentrations were maintained, liver reserves did not differ from children who ate white maize. The concentration of provitamin A carotenoids was about 6 μg/g. Therefore, efforts to achieve the preliminary target level of 15 μg/g should continue, which would provide the estimated average requirement of vitamin A to children who consume 200 g dry maize/day [78].

Scaling up nutrition through biofortification requires scaling up agriculture

The World Bank has estimated that US$ 11.8 billion is needed per year to scale up proven nutrition interventions [83]. Three broad nutrition interventions have been discussed including: 1) behavior change, 2) micronutrient and deworming interventions, and 3) complementary and therapeutic feeding [83]. While certainly each of these interventions is important in the maintenance of child health among the undernourished, they all rely on outside and continuous resources. Surprisingly, none of the proposed interventions include enhanced agriculture through biofortification, which is a sustainable and potentially effective component of a nutrition and health improvement strategy.

Good health through nutrition and productive agriculture are essential in the fight against poverty. Agriculture affects nutrition and health, and health affects agricultural productivity (Table 3). Health problems cut productivity and income, leading to persistent poverty, reduced food security, and stalled economic development [84] (Figure 5). Agricultural development requires front-loaded and long-term investments, but usually has low recurrent costs [6]. Improving agriculture to provide more nutritious foods through a sustainable intervention is key to improving the health of malnourished populations and should be part of scaling up nutrition strategies [22]. Biofortification of staple crops with provitamin A is just one example of what needs to be accomplished.

Impacting food insecurity and hunger

Solving hidden hunger through biofortification may require two generations to see outcomes, especially with provitamin A carotenoids, due to bioavailability issues and whole body regulation. For example, a woman who starts eating a biofortified food during pregnancy may not transfer much of the vitamin A to the fetus if she herself is vitamin A-depleted. During the lactation period, continued consumption of the biofortified food will certainly enhance liver reserves of vitamin A in the newborn, but it may not be enough to bring the child into an adequate status if the infant undergoes repeated infections. However, if these children continue to consume the biofortified food throughout childhood, into adolescence, and into the reproductive years, the next generation will start with a better overall status, which will translate to better stores in the fetus and newborn [85]. Anecdotal evidence in a recent study with Zambian children revealed that the lactating women who were "watching" the trial and partaking of the leftover food after the children ate, realized that their nursing infants were also benefiting from the intervention. Nutrition and health education are keys to moving forward the complex agenda of solving hidden hunger. Integrated approaches, which may include supplementation, fortification, and biofortification, need to be considered in the short- and medium-term, with the ultimate goal of improved, nutritionally balanced diets in the long-term.

Biofortification: What really matters?

Committed team approaches which include multiple disciplines are needed to support scaled-up nutrition and agriculture to make lasting impacts on hunger and micronutrient deficiencies through crop enhancement. The resultant high-yielding biofortified staple crops can contribute to improved nutritional status, improved health status, increased work capacity, and enhanced income. The enhanced provitamin A carotenoids in staple crops are bioavailable and an impact on vitamin A status is on the horizon in those populations that consume provitamin A-biofortified staple foods.

Conclusions

In the interim, integrated approaches which support the nutritional needs of target populations are needed. Vitamin A supplementation causes recurring fluctuations in liver vitamin A reserves, and rarely causes chronic toxicity [5, 86]. Fortification of commonly consumed foods with pre-formed vitamin A, on the other hand, does have this toxicity potential [5, 36]. This difference is due in part to the chemical forms in which vitamin A is found in supplements, which are oil-based, and fortificants, which are usually water-miscible; in a meta-analysis, water-miscible forms of pre-formed vitamin A were linked to increased toxicity [87]. Food-based approaches with provitamin A sources, such as biofortified staple crops, which typically constitute a disproportionately large portion of resource-poor household diets, have the potential to improve vitamin A status without fluctuating liver reserves or risk of toxicity [5, 86]. Promoting provitamin A sources alongside of supplementation and fortification efforts is safe and desirable to achieve the goal of eradicating hidden hunger among those at risk for vitamin A deficiency.

Acknowledgment

Supported by HarvestPlus contract numbers 5204, 6210, 8217, and 8202. Financial support for this study was provided by HarvestPlus (www.HarvestPlus.org), a global alliance of agriculture and nutrition research institutions working to increase the micronutrient density of staple food crops through biofortification. The views expressed do not necessarily reflect those of HarvestPlus.

References

1. WHO (World Health Organization). (1995) Vitamin A deficiency and its consequences: a field guide to detection and control (third edition). Available: http://www.who.int/nutrition/publications/micronutrients/vitamin_a_deficieny/9241544783/en/index.html. Accessed November 2010.

2. Sun, T., Surles, R.L. and Tanumihardjo, S.A. (2008) Vitamin A concentrations in piglet extrahepatic tissues respond differently 10 d after vitamin A treatment. J. Nutr. 138, 1101–1106.

3. Penniston, K.L. and Tanumihardjo, S.A. (2001) Subtoxic hepatic vitamin A concentrations in captive rhesus monkeys (*Macaca mulatta*). J. Nutr. 131, 2904–2909.

4. Tanumihardjo, S.A., Anderson, C., Kaufer-Horwitz, M., Bode, L., Emenaker, N.J., Haqq, A.M., Satia, J.A., Silver, H.J. and Stadler, D.D. (2007) Poverty, obesity and malnutrition: an international perspective recognizing the paradox. J. Am. Diet. Assn. 107, 1966–1972.

5. Tanumihardjo, S.A. (2008) Food-based approaches for ensuring adequate vitamin A nutrition. Comp. Rev. Food Sci. Food Safety 7, 373–381.

6. Tanumihardjo, S.A., Bouis, H., Hotz, C., Meenakshi, J.V. and McClafferty, B. (2008) Biofortification of staple crops: An emerging strategy to combat hidden hunger. Comp. Rev. Food Sci. Food Safety 7, 329–334.

7. De Pee, S. and West, C.E. (1996) Dietary carotenoids and their role in combating vitamin A deficency: a review of the literature. Eur. J. Clin. Nutr. 50 (Suppl.3), S38–53.

8. Castenmiller, J.J.M. and West, C.E. (1998) Bioavailability and bioconversion of carotenoids. Annu. Rev. Nutr. 18, 19–38.

9. Furr, H.C., Green, M., Haskell, M., Mokhtar, N., Nestel, P., Newton, S., Ribaya-Mercado, J., Tang, G., Tanumihardjo, S.A. and Wasantwisut E. (2005) Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. Public Health Nutr. 8, 596–607.

10. de Pee, S., West, C.E., Muhilal, Karyadi, D. and Hautvast, J.G. (1995) Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 346, 75–81.

11. Parker, R.S. (1996) Absorption, metabolism, and transport of carotenoids. FASEB J. 10, 542–551.

12. Canfield, L.M., Fritz, T.A. and Tarara, T.E. (1990) Incorporation of β-carotene into mixed micelles. Methods Enzymol. 189, 418–422.

13. Tanumihardjo, S.A. (2002) Factors influencing the conversion of carotenoids to retinol: Bioavailability to bioconversion to bioefficacy. Int. J. Vitam. Nutr. Res. 72, 40–45.

14. Van Lieshout, M., West, C.E., Muhilal, Permaesih, D., Wang T., Xu, X. van Breemen, R.B., Creemers, A.F.L., Verhoeven, M.A. and Lugtenburg, J. (2001) Bioefficacy of β-carotene dissolved in oil studied in children in Indonesia. Am. J. Clin. Nutr. 73, 949–958.

15. De Pee, S., West, C.E., Permaesih, D., Martuti, S., Muhilal and Hautvast, J.G.A.J. (1998) Orange fruit is more effective than are dark-green leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. Am. J. Clin. Nutr. 68: 1058–1067.

16. National Research Council (1989) Recommended Dietary Allowances, 10th edition. National Academy Press, Washington, D.C.

17. Institute of Medicine. (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. pp. 65–126, National Academy Press, Washington, D.C.

18. FAO (Food and Agricultural Organization of the United Nations). (2001) Chapter 7: Vitamin A. In: Human vitamin and mineral requirements. Available: ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e07.pdf. Accessed November 9, 2010.

19. Li, S., Nugroho, A., Rocheford, T. and White, W.S. (2010) Vitamin A equivalence of the β-carotene in β-carotene biofortified maize porridge consumed by women. Am. J. Clin. Nutr. 92, 1105–1112.

20. Bouis, H.E. (2002) Plant breeding: a new tool for fighting micronutrient malnutrition. J. Nutr. 132, 491S-494S.

21. Hirschi, K.D. (2009) Nutrient biofortification of food crops. Annu. Rev. Nutr. 29, 401–421.

22. Meenakshi, J.V. (2009) Best practice paper: Cost-effectiveness of biofortification. Copenhagen Consensus Center, Frederiksberg, Denmark. www.copenhagenconsensus.com.

23. Pfeiffer, W.H. and McClafferty, B. (2007) HarvestPlus: Breeding crops for better nutrition. Crop Sci. 47, S88-S105.

24. Tanumihardjo, S.A. and Howe, J.A. (2005) Twice the amount of α-carotene isolated from carrots is as effective as β-carotene in maintaining the vitamin A status of Mongolian gerbils. J. Nutr. 135, 2622–2626.

25. Davis, C.R., Jing, H., Howe, J.A., Rocheford, T.R. and Tanumihardjo, S.A. (2008) β-Cryptoxanthin from supplements or carotenoid enhanced maize maintains liver vitamin A in Mongolian gerbils (*Meriones unguiculatus*) better than or equal to β-carotene supplements. Br. J. Nutr. 100, 786–793.

26. Davis, C.R., Howe, J.A., Rocheford, T.R. and Tanumihardjo, S.A. (2008) The xanthophyll composition of biofortified maize (*Zea mays sp.*) does not influence the bioefficacy of provitamin A carotenoids in Mongolian gerbils (*Meriones unguiculatus*). J. Agric. Food Chem. 56, 6745–6750.

27. Howe, J.A. and Tanumihardjo, S.A. (2006) Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. J. Nutr. 136, 2562–2567.

28. Muzhingi, T., Tang, G., Siwela, A., Gadaga, H.T. and Grusak, M. (2010) Vitamin A value of high beta-carotene yellow maize in humans. FASEB J. 24, 103.1.

29. Thakkar, S.K. and Failla, M.L. (2008) Bioaccessibility of pro-vitamin A carotenoids is minimally affected by non pro-vitamin A xanthophylls in maize (*Zea mays* sp.). J. Agric. Food Chem. 56, 11441–11446.

30. Howe, J.A., Maziya-Dixon, B. and Tanumihardjo, S.A. (2009) Cassava with enhanced β-carotene maintains adequate vitamin A status in Mongolian gerbils (*Meriones unguiculatus*) despite substantial cis-isomer content. Br. J. Nutr. 102, 342–349.

31. Deming, D.M., Teixeira, S.R. and Erdman, J.W. Jr. (2002) All-trans β-carotene appears to be more bioavailable than 9-cis or 13-cis β-carotene in gerbils given single oral doses of each isomer. J. Nutr. 132, 2700–2708.

32. Deming, D.M., Baker, D.H. and Erdman, J.W. Jr. (2002) 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. 132, 2709–2712.

33. Bresnahan, K., Davis, C.R. and Tanumihardjo, S.A. (2011) The relative vitamin A value of 9-*cis*- and 13-*cis*-β-carotene did not differ in vitamin A-depleted Mongolian gerbils (*Meriones unguicalatus*). FASEB J. (In press).

34. Leitz, G., Lange, J. and Rimbach, G. (2010) Molecular and dietary regulation for β, β-carotene 15, 15'-monooxygenase 1 (BCMO1). Arch. Biochem. Biophys. 502, 8–16.

35. Ribaya-Mercado, J.D., Solon, F.S., Solon, M.A., Cabal-Barza, M.A., Perfecto, C.S., Tang, G., Solon, J.A.A., Fjeld, C.R. and Russell, R.M. (2000) Bioconversion of plant carotenoids to vitamin A in Filipino school-aged children varies inversely with vitamin A status. Am. J. Clin. Nutr. 72, 455–465.

36. Ribaya-Mercado, J.D., Solomons, N.W., Medrano, Y., Bulux, J., Dolnikowski, G.G., Russell, R.M. and Wallace, C.B. (2004) Use of the deuterated-retinol-dilution technique to monitor the vitamin A status of Nicaraguan schoolchildren 1 y after initiation of the Nicaraguan national program of sugar fortification with vitamin A. Am. J. Clin. Nutr. 80, 1291–1298.

37. Suharno, D., West, C.E., Muhilal, Karyadi, D. and Hautvast, J.G. (1993) Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. Lancet 342, 1325–1328.

38. Ahmed, F., Khan, M.R. and Jackson, A.A. (2001) Concomitant supplemental vitamin A enhances the response to weekly supplemental iron and folic acid in anemic teenagers in urban Bangladesh. Am. J. Clin. Nutr. 74, 108–115.

39. Muslimatun, S., Schmidt, M.K., Schultink, W., West, C.E., Hautvast, J.A., Gross, R. and Muhilal (2001) Weekly supplementation with iron and vitamin A during pregnancy increases hemoglobin concentration but decreases serum ferritin concentration in Indonesian pregnant women. J. Nutr. 131, 85–90.

40. Tanumihardjo, S.A. (2002) Vitamin A and iron status are improved with vitamin A and iron supplementation in pregnant Indonesian women. J. Nutr. 132, 1909–1912.

41. Semba, R.D. and Bloem, M.W. (2002) The anemia of vitamin A deficiency: epidemiology and pathogenesis. Eur. J. Clin. Nutr. 56, 271–281.

42. Dijkhuizen, M.A., Wieringa, F.T., West, C.E. and Muhilal (2004) Zinc plus β-carotene supplementation of pregnant women is superior to β-carotene supplementation alone in improving vitamin A status in both mothers and infants. Am. J. Clin. Nutr. 80, 1299–1307.

43. Christian, P. and West, K.P., Jr. (1998) Interactions between zinc and vitamin A: an update. Am. J. Clin. Nutr. 68, 435S-441S.

44. Sundaresan, P.R., Cope, F.O. and Smith, J.C., Jr. (1977) Influence of zinc deficiency on retinal reductase and oxidase activities in rat liver and testes. J. Nutr. 107, 2189–2197.

45. Ribaya-Mercado, J.D., Maramag, C.C., Tengco, L.W., Dolnikowski, G.G., Blumberg, J.B. and Solon, F.S. (2007) Carotene-rich plant foods ingested with minimal dietary fat enhance the total-body vitamin A pool size in Filipino schoolchildren as assessed by stable-isotope-dilution methodology. Am. J. Clin. Nutr. 85, 1041–1049.

46. Mills, J.P., Tumuhimbise, G., Jamil, K.M., Thakkar, S., Failla, M.L. and Tanumihardjo, S.A. (2009) Sweet potato β-carotene bioefficacy is enhanced by dietary fat and not reduced by soluble fiber intake in Mongolian gerbils. J. Nutr. 139, 44–50.

47. Chitchumroonchokchai, C., Failla, M.L., Ferruzzi, M.G. and Campbell, W.W. (2010) Dietary fats with increased ratio of unsaturated to saturated fatty acids enhance absorption of carotenoid and vitamin E by increasing both efficiency of micellarization and lipoprotein secretion. FASEB J. 24, 539.3.

48. Porter Dosti, M., Mills, J.P., Simon, P.W. and Tanumihardjo, S.A. (2006) Bioavailability of β-carotene (βC) from purple carrots is the same as typical orange carrots while high-βC carrots increase βC stores in Mongolian gerbils (*Meriones unguiculatus*). Br. J. Nutr. 96, 258–267.

49. USDA National Nutrient Database for standard reference. Available: http://www.nal.usda.gov/fnic/foodcomp/search/. Accessed November 19, 2010.

50. Liu, W., Zhou, Y., Sanchez, T., Ceballos, H., and White, W.S. (2010) The vitamin A equivalence of β-carotene in β-carotene-biofortified cassava ingested by women. FASEB J. 24, 92.7.

51. Tang, G., Qin, J., Dolnikowski, G.G., Russell, R.M. and Grusak, M.A. (2009) Golden Rice is an effective source of vitamin A. Am. J. Clin. Nutr. 89, 1776–1783.

52. Tang, G., Yin, S.-A., Wang, Y., Grusak, M. and Russell, R. (2010) Provitamin A from Golden Rice is as effective as provitamin A in oil to children. FASEB J. 24, 537.24.

53. Haskell, M.J., Jamil, K.M., Hassan, F., Peerson, J.M., Hossain, M.I., Fuchs, G.J. and Brown, K.H. (2004) Daily consumption of Indian spinach (*Basella alba*) or sweet potatoes has a positive effect on total-body vitamin A stores in Bangladeshi men. Am. J. Clin. Nutr. 80, 705–714.

54. van Jaarsveld, P.J., Faber, M., Tanumihardjo, S.A., Nestel, P., Lombard, C.J. and Benade, A.J. (2005) β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. Am. J. Clin. Nutr. 81, 1080–1087.

55. Low, J.W., Arimond, M., Osman, N., Cunguara, B., Zano, F. and Tschirley, D. (2007) A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. J. Nutr. 137,1320–1327.

56. Low, J.W. and van Jaarsveld, P.J. (2008) The potential contribution of bread buns fortified with beta-carotene-rich sweet potato in Central Mozambique. Food Nutr. Bull. 29, 98–107.

57. Paine, J.A., Shipton, C.A., Chaggar, S., Howells, R.M., Kennedy, M.J., Vernon, G., Wright, S.Y., Hinchliffe, E., Adams, J.L., Silverstone, A.L. and Drake, R. (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat. Biotechnol. 23, 482–487.

58. Arscott, S.A., Howe, J.A., Davis, C.R. and Tanumihardjo, S.A. (2010) Carotenoid profiles in provitamin A-containing fruits and vegetables affect the bioefficacy in Mongolian gerbils. Exp. Biol. Med. (Maywood). 235, 839–848.

59. Davey, M.W., Garming, H., Ekesa, B., Roux, N. and Van den Bergh, I. (2007) Exploiting banana biodiversity to reduce vitamin A deficiency related illness: a fast and cost-effective strategy. J. Agric. Food Chem. 55, 2633–2644.

60. Bresnahan, K., Arscott, S.A. and Tanumihardjo, S.A. (2011) Vitamin A bioefficacy of traditional and provitamin A-biofortified banana. FASEB J. (In press).

61. Ejoh, R.A., Dever, J.T., Mills, J.P. and Tanumihardjo, S.A. (2010) Small quantities of carotenoid-rich tropical green leafy-vegetables indigenous to Africa maintain vitamin A status in Mongolian gerbils (*Meriones unguiculatus*). Br. J. Nutr. 103, 1594–1601.

62. Tanumihardjo, S.A. (2001) Can lack of improvement in vitamin A status indicators be explained by little or no overall change in vitamin A status of humans? J. Nutr. 131, 3316–3318.

63. Tang, G., Gu, X., Hu, S., Xu, Q.-M., Qin, J., Dolnikowski, G.G., Fjeld, C.R., Gao, X., Russell, R.M. and Yin, S.-A. (1999) Green and yellow vegetables can maintain body stores of vitamin A in Chinese children. Am. J. Clin. Nutr. 70, 1069–1076.

64. Kang, W. and Kudsk, K.A. (2007) Is there evidence that the gut contributes to mucosal immunity in humans? J. Parenter. Enteral. Nutr. 31, 246–258.

65. Latham, M. (2010) The great vitamin A fiasco. World Nutr. 1, 12–45.

66. Beaton, G.H., Martorell, R. and L'Abbé, K.A. (1993) Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries: Summary report. University of Toronto, Toronto, Canada.

67. Neidecker-Gonzales, O., Nestel, P. and Bouis, H. (2007) Estimating the global costs of vitamin A capsule supplementation: A review of the literature. Food Nutr. Bull. 28, 307–316.

68. Mills, J.P., Simon, P.W. and Tanumihardjo, S.A. (2008) Biofortified carrot intake enhances liver antioxidant capacity and vitamin A status in Mongolian gerbils. J. Nutr. 138, 1692–1698.

69. MyPyramid.gov [homepage on the Internet]. Washington, D.C.: United States Department of Agriculture Center for Nutrition Policy and Promotion [released 2005 Apr]. Available from: http://www.mypyramid.gov/.

70. Tanumihardjo, S.A., Valentine, A.R., Zhang, Z., Whigham, L.D., Lai, H.J. and Atkinson, R.L. (2009) Strategies to increase vegetable or reduce energy and fat intake induce weight loss in adults. Exp. Biol. Med. 234, 542–552.

71. Welch, R. M. (2002) Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J. Nutr. 132, 495S-499S.

72. United Nations Department of Economic and Social Affairs. The Millennium Development Goals Report. New York, 2006.

73. United Nations Department of Economic and Social Affairs. The Millennium Development Goals Report. New York, 2010.

74. World Bank. The World Development Report 2008: Agriculture for Development. World Bank, Washington, D.C., 2008.

75. Spielman, D.J. and Pandya-Lorch, R. (2009) Millions fed: Proven successes in agricultural development. International Food Policy Research Institute, Washington, D.C.

76. Welch, R.M. and Graham, R.D. (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J. Exp. Bot. 55, 353–364.

77. Nuss, E.T. and Tanumihardjo, S.A. (2010) Maize: A paramount staple crop in the context of global nutrition. Comp. Rev. Food Sci. Food Saf. 9, 417–436.

78. Harvest Plus. (2007) Biofortified maize crops for better nutrition. Retrieved July, 2010 from http://crop.scijournals.org/cgi/reprint/47/Supplement_3/S-88.

79. Thakkar, S.K. and Failla, M.L. (2008) Bioaccessibility of pro-vitamin A carotenoids is minimally affected by non pro-vitamin A xanthophylls in maize (*Zea mays* sp.). J. Agric. Food Chem. 56, 11441–11446.

80. Kean, E.G., Hamaker, B.R. and Ferruzzi, M.G. (2008) Carotenoid bioaccessibility from whole grain and degermed maize meal products. J. Agric. Food Chem. 56, 9918–9926.

81. Li, S., Tayie, F.A.K., Young, M.F., Rocheford, T. and White, W.S. (2007) Retention of provitamin A carotenoids in high-β-carotene maize (*Zea mays*) during traditional African household processing. J. Agric. Food Chem. 55, 10744–10750.

82. Nuss, E.T., Arscott, S.A., Bresnahan, K., Pixley, K.V., Rocheford, T., Hotz, C., Siamusantu, W., Chileshe, J. and Tanumihardjo, S.A. (2011) Adaptation to and intake patterns of traditional foods made from biofortified orange maize (*Zea mays*) in rural Zambian children. FASEB J. (In press).

83. Horton, S., Shekar, M., McDonald, C., Mahal, A. and Brooks, J.K. (2010) Scaling up nutrition: What will it cost? The World Bank: Washington, D.C.

84. Hawkes, C. and Ruel, M. (2006) Understanding the links between agriculture and health. IFPRI 2020 vision focus briefs.

85. Mills, J.P., Terasawa, E. and Tanumihardjo, S.A. (2007) Excessive preformed vitamin A intake by mothers amplifies early fetal liver retinyl ester storage in captive Old World monkeys. Comp. Med. 57, 505–511.

86. Allen, L.H. and Haskell, M. (2002) Estimating the potential for vitamin A toxicity in women and young children. J. Nutr. 132, 2907S-2919S.

87. Myhre, A.M., Carlsen, M.H., Bohn, S.K., Wold, H.L., Laake, P. and Blomhoff, R. (2003) Water-miscible, emulsified, and solid forms of retinol supplements are more toxic than oil-based preparations. Am. J. Clin. Nutr. 78, 1152–1159.

Sherry A. Tanumihardjo

UW-Madison
1415 Linden Drive
Madison, Wi 53706
USA
Phone: 608–265–0792
Fax: 608–262–5860
E-mail: sherry@nutrisci.wisc.edu