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Influence of Provitamin A Carotenoids on Iron, Zinc, and Vitamin A Status

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Introduction

Bioavailable iron, vitamin A, and zinc are mainly provided in the human diet by animal source foods. In the developing world, where poorer individuals consume predominantly plant-based diets, deficiencies of these micronutrients are common and can occur in the same individual. Infants, children, and pregnant and lactating women are most at-risk of deficiency because of their extra requirements for growth. About 250 million preschool children are estimated to be vitamin A deficient based on low serum retinol values, although the real figure may be lower because serum retinol is depressed by inflammation. Women of reproductive age are especially at risk of iron deficiency because of the blood loss associated with menstruation. On the basis of low serum ferritin concentrations, which indicate low iron stores, it is estimated that about 2 billion people worldwide are iron deficient (WHO 2004). However, this estimate may be too low because serum ferritin is increased by inflammation. Approximately one-half of the 2 billion people showing low ferritin concentrations additionally show low hemoglobin (or other iron status measures outside the normal range), suggesting that they suffer from iron deficiency anemia (IDA), the most severe form of iron deficiency. The exact prevalence of zinc deficiency is not known due to the lack of an effective measure of zinc status. However, based on factors influencing dietary intake and absorption as well as extra growth requirements, the prevalence of zinc deficiency is often proposed as being similar to that of iron deficiency.

The co-occurrence of iron and vitamin A deficiencies has been found in infants in South Africa (Oelofse et al. 2002), preschool children in the Marshall Islands (Palafox et al. 2003) and Honduras (Albalak et al. 2000), school-age children in Côte d'Ivoire (Hess 2003), and pregnant women in India (Pathak et al. 2003), Nepal (Dreyfuss et al. 2000), and Malawi (van den Broek and Letsky 2000). In the Côte d'Ivoire, for example, 10% of 6- to 15-year-old children had IDA and vitamin A deficiency (serum retinol < 0.7 μmol/L) (Hess 2003), and in Malawi 17% of pregnant women had both IDA and indications of low vitamin A stores (serum retinol < 1.05 μmol/L) (van den Broek and Letsky, 2000).

When an individual is deficient in more than one nutrient, interactions and/or a causative factor responsible for both deficiencies can exist and a deficiency in one nutrient can influence the utilization of a second nutrient. For example, when vitamin A is deficient in the diet, iron metabolism is negatively affected and iron is not incorporated effectively into hemoglobin (Hodges et al. 1978). An interaction between vitamin A and zinc metabolism has also been suggested (Christian and West 1998).

In the context of plant breeding and biotechnology to enhance the levels of—or improve the bioavailability of—iron, provitamin A carotenoids, and zinc to combat micronutrient malnutrition, this review discusses in detail the influence of vitamin A status on iron utilization and the consequent impact on iron status. The much less extensive literature on the interaction between vitamin A and zinc metabolism is also presented and discussed. Prior to these discussions, recent reports on the efficacy of the bioconversion of provitamin A carotenoids into retinol, and the influence of these carotenoids on vitamin A status, are presented and evaluated. An attempt is made to define the concentrations of provitamin A carotenoids that might have a significant impact on vitamin A status and to evaluate the usefulness or necessity of co-biofortifying iron- or zinc-enriched staple foods with β-carotene. Finally, knowledge gaps are identified and future research topics are proposed.

Effect of β-carotene on vitamin A status

There are two forms of vitamin A in foods: preformed retinol as retinyl esters and provitamin A carotenoids. The three major provitamin A carotenoids are β-carotene, which consists of two molecules of retinol joined together, and α-carotene and β-cryptoxanthin, which consist of one retinol molecule joined to another retinoid with no vitamin A activity. Preformed retinol from animal source foods, such as liver, eggs, and dairy products is the most bioavailable dietary source of vitamin A; however, provitamin A carotenoids from plant foods can be hydrolyzed in the intestinal mucosa

to liberate retinol. In fact, plant source foods provide much of the vitamin A intake of poorer individuals living in developing countries. β-carotene is the major provitamin A carotenoid, and theoretically enzymatic cleavage of one β-carotene molecule generates two molecules of retinal, compared with only one retinal molecule on cleavage of all other provitamin A carotenoids. The retinal is further converted to retinol and then to retinoic acid as required. However, the absorption of provitamin A carotenoids is influenced by various factors (West and Castenmiller 1998) and their efficacy in improving vitamin A status was recently found to be much lower than had been previously thought (Institute of Medicine 2001).

Retinyl esters ingested with food are solubilized in the lumen into micelles together with hydrolytic products of triglycerides, phospholipids, and bile acids, and converted to retinol that is about 70–90% absorbed (Sivakumar and Reddy 1972). A specific transport protein facilitates retinol uptake (Dew and Ong 1994). Carotenoids are also solubilized within micelles but are absorbed by passive diffusion. Absorption of a single dose of β-carotene has been reported to vary from 4–55% (Goodman et al. 1966; Blomstrand and Werner 1967; Hickenbottom et al. 2002a; Hickenbottom et al. 2002b); however many additional factors affect the bioavailability of carotenoids from foods (Castenmiller and West 1998). Individuals with lower vitamin A status appear to have higher absorption and/or bioconversion of carotenoids (Ribaya-Mercado et al. 2000). Absorption varies greatly depending on the plant matrix and is increased by the presence of oil and by heat processing (Rock et al. 1998). For example, consumption of heat processed and pureed spinach and carrots resulted in a 3-fold increase in plasma β-carotene conversion compared with consumption of uncooked portions of these vegetables. Absorbed provitamin A carotenoids are mainly cleaved enzymatically in intestinal mucosal cells and retinol is esterified. Retinyl esters and nonhydrolyzed carotenoids are packaged into chylomicrons with newly digested lipid and transported to the liver.

The vitamin A activity of provitamin A carotenoids was for many years expressed as retinol equivalents (RE) (FAO/WHO Expert Consultation 1988). Based on more recent absorption and bioconversion data, however, it is now recommended that provitamin A activity in foods be expressed as retinol activity equivalents (RAE), which are 50% of the corresponding RE (Institute of Medicine 2001). RE was defined based on the assumption that 33% of β-carotene in food is absorbed and that 50% is converted to retinol. The definition of RAE, by contrast, is based on the assumption that 16.7% of the β-carotene is absorbed and 50% is converted to retinol. However, RAE should still be viewed as an approximate guideline because it was defined on the basis of relatively few studies and the absorption and conversion of β-carotene from different foods has been reported to vary by up to five fold. RAE values also take into account the observation that the carotene to retinol equivalency ratio (wt:wt) of the low dose of β-carotene required to correct night blindness is approximately 2:1 (Sauberlich et al. 1974), which suggests that when retinol is needed, the combined absorption and bioconversion of free synthetic β-carotene in oil to retinol is about 50% as effective as giving retinol alone. Most studies have used the increase in plasma β-carotene after a meal as a measure of absorption and reported β-carotene absorption to vary from 4% in mixed green leafy vegetables (de Pee et al. 1995), 5% in spinach (Castenmiller et al. 1999), 11–12% in broccoli (Micozzi et al. 1992), to 18–41% in carrots (Micozzi et al. 1992; Törrönen et al. 1996; Livny et al. 2003).

Using the increase in plasma β-carotene concentration to measure absorption is, by definition, guaranteed to underestimate the true absorption. The β-carotene that enters the plasma following a meal is the fraction of absorbed β-carotene that was not converted to retinol in the intestinal cells. In addition, studies in rats have shown that conversion of β-carotene to retinol is higher if the rats are vitamin A deficient and lower if they are protein deficient (Parvin and Sivakumar 2000). Thus, nutritional status also affects the variability in β-carotene bioavailability from different foods, further complicating any attempt to interpret absorption from plasma β-carotene concentrations.

The approach of using changes in plasma retinol concentration to measure absorption of β-carotene from foods is also unreliable unless precautions are taken to control for changes in subclinical inflammation. To date, only one study has used the increase in serum retinol to measure vitamin A activity; it found that β-carotene in orange tubers, such as squash, and orange fruits had approximately double the vitamin A activity of β-carotene in dark green leafy vegetables (de Pee et al. 1998). The study was done in 7 to 11 year old rural Indonesian children who were fed one of four diets, twice a day for 9 weeks. Two diets were rich in β-carotene from either a vegetable or a fruit source, one was rich in retinol, and the fourth was low in both retinol and β-carotene. The plasma retinol concentration in all four groups was ~0.7 μmol/L at the start, which is low for children of this age. The largest increase in plasma retinol concentration (~0.23 μmol/L) was observed in the group that received vitamin A in the form of retinol, but no attempt was made to treat the intestinal parasites that affected ~80% of the children. Other studies in this district have shown a high prevalence of inflammation or immunological activity in similar populations (Wieringa et al. 2002; Wieringa et al. 2004). Given the high likelihood that plasma retinol concentration will be depressed in individuals with inflammation (Thurnham et al. 2003), the true response to the vitamin A intake in this study was probably underestimated. There have been no direct comparisons of the vitamin A activity of dietary provitamin A carotenoids and preformed retinol in either healthy individuals or subjects from developing countries where micronutrient deficiencies, chronic infections, and inflammation have been identified or quantified.

Conversion factors are needed to calculate RAE values based on the carotenoid contents of diets and individual foods. However, given the limited data currently available, estimates of these conversion factors are still approximations and may change as new information comes available. This should be borne in mind when using these factors to plan and evaluate biofortification programs to increase intake of carotenoids. The current factors proposed by the US Institute of Medicine (2001) are that 1 RAE is equivalent to 1 μg of preformed retinol, 2 μg of supplemental β-carotene in oil, 12 μg of dietary β-carotene, and 24 μg of other provitamin A carotenoids.

Cross-sectional and case-control studies

Cross-sectional and case-control studies have demonstrated the importance of dietary provitamin A carotenoids from plant foods in preventing vitamin A deficiency, but have also highlighted the superiority of preformed retinol from animal foods in maintaining vitamin A status. Several cross-sectional studies in young children have shown an association between increased risk of xerophthalmia and less frequent consumption of carotene-rich vegetables and fruits (Table 1, p. 7) (Pepping et al. 1989; Mele et al. 1991; Nestel et al. 1993; Schaumberg et al. 1996), although other studies, such as one conducted in Bangladesh (Stanton et al. 1986), have found that the risk of xerophthalmia is not related to fruit and vegetable intake, but is lowered by higher consumption of milk and eggs. Similarly in Nepal, dietary assessments in case control studies have indicated that dietary intake of preformed vitamin A from animal foods may be more important than carotenoid-containing plant food consumption in protecting children and other family members from vitamin A deficiency (Shankar et al. 1996). No significant correlation between frequency of vegetable consumption and either plasma retinol or plasma carotenoids was observed in pregnant women in Tanzania (Mulokozi et al. 2003). However, although the mean plasma retinol concentrations (0.89 μmol/L) were low in the Tanzanian women, plasma lutein and β-carotene concentrations were 1.61 and 0.63 μmol/L, respectively, indicating that a large amount of carotene was absorbed. Given that the amount of β-carotene in the plasma represents the fraction not converted to retinol, there was adequate dietary β-carotene to convert to retinol. In other words, other factors were probably depressing the plasma retinol concentration and obscuring the association between plasma retinol concentrations and the amount of vitamin A consumed.

The vitamin A concentration in breast milk is closely related to the plasma retinol concentration of the mother (de Pee et al. 1997; Dijkhuizen et al. 2001) and adequate vitamin A intake is crucial to the health of

TABLE 1

Factors associated with xerophthalmia in different countries

Country	Diet staples	Age	Xerophthalmia		Reference
			Factors associated	Other interesting/related points	
Rural Tanzania	Maize, sweet potato, sorghum, cassava	4–9 years	Intake of β-carotene, folic acid, and iron especially low	20% of total β-carotene from dried GLV*	Pepping et al. 1989
Aceh, Indonesia	Rice	< 3 years	Low frequency of intake of dark GLV*, yellow fruits, and eggs	Associated with high risk of dietary imbalance	Mele et al. 1991
N. Sudan	Wheat	6–72 months	Living in remote and arid regions, male gender, young age, poverty of household, and diarrhea	Low risk of X** in children consuming dairy products in previous 24 hr	Nestel et al. 1993
Republic of Kiribati, South Pacific	Most common weaning foods: breadfruit, papaya, green coconut, fish	Pre-school children	When controlled for age and sex, X** associated with diarrhea (OR 1.45) and wasting (OR >3.0). Protective factors were breast feeding (OR 0.3), food rich in VA# (OR 0.93) and presence of garden project (OR 0.7)	A recent history of measles associated with corneal X	Schaumberg et al. 1996
Urban Bangladesh	Rice	< 14 years	After controlling for demographic characteristics, male gender, greater age (mean 6.1 years), large family size, poor intake of local VA# rich foods, and recent history of protracted diarrhea	Maternal ignorance of prevention and control of vitamin A deficiency associated with increased risk	Stanton et al. 1986
Rural Nepal	Rice	1–6 years	Infrequent intake of β-carotene and preformed VA# rich food clusters among siblings within household; more common in rice harvest season (October to December)	Control households more likely than cases to consume VA# rich foods during monsoon and rice harvest	Shankar et al. 1996

* GLV green leafy vegetables; ** X, xerophthalmia; # VA vitamin A

both mother and infant, indicating that factors other than dietary intake of carotene-containing foods are also important in influencing plasma retinol concentrations. Nevertheless, the studies cited above indicate that in many developing countries, low intake of provitamin A carotenoids from plant foods is often associated with an increased risk of xerophthalmia.

Intervention studies

Intervention studies investigating the effect of increased consumption of β-carotene from food on vitamin A status in developing countries have yielded equivocal results, probably due to the many factors affecting outcome. Although several community-based intervention studies suggest the vitamin A status of a population can be improved by promoting cultivation and/or consumption of carotene-rich foods, de Pee and West (1996) argued that the lack of a control group in most of these studies made it difficult to draw conclusions on the usefulness of this practice. Another problem in the older literature is that many studies quantified food-derived carotenes in RE values (i.e., where 1 μg RE equals 6 μg β-carotene) whereas the more recently introduced RAE values are 50% lower (1 μg RAE equals 12 μg β-carotene). However, no change has occurred with respect to the RE and RAE equivalency for retinol or β-carotene in oil. This may partly explain why daily supplementation with dark green leafy vegetables of the diet of lactating Indonesian women with low vitamin A status did not appear to increase vitamin A status, whereas supplementation with the same amount of β-carotene given in an oil matrix on a wafer led to a significant increase in plasma retinol and a reduction in the dehydroretinol/retinol (DR/R) ratio (de Pee et al. 1995). However, even assuming that the amounts of bioavailable β-carotene in the vegetables and the wafer were not the same, other factors also need to be considered in interpreting this study. Stool egg counts indicated that ≥80% of the women had ascaris and/or trichuris parasites and ~25% were infected with *Entamoeba hystolytica*. The women were not dewormed before the study and subclinical inflammation was not assessed. Serum albumin concentrations and leukocyte counts showed little variation from normal values, but these are not as sensitive indicators of inflammation as acute phase proteins like C-reactive protein or a1-acid glycoprotein (Fleck and Myers 1985). Thus inflammation

may have influenced both the changes in plasma retinol concentration (Thurnham and Singkamani 1991), as well as the DR/R ratio (Stephensen et al. 2002).

There is some evidence, however, that consumption of high β-carotene containing foods increases vitamin A status, with fresh fruits seemingly being better than vegetables at providing bioavailable provitamin A carotenoids. In a 9 week study in anemic school children in Indonesia, for example, the authors claimed that the increase in serum retinol concentration associated with consuming fruit was twice that achieved from consuming vegetables (de Pee et al. 1998) (Table 2, p. 9). Moreover, the increase in serum β-carotene in relation to the amount of β-carotene provided was 5–6 times higher for fruit than for vegetables. Other studies, however, have been less successful. A study in Gambian children compared the effects of daily supplementation of freshly rehydrated mango containing approximately 150 μg RAE with or without 5 g of additional fat, for 5 days each week, over four months. Two other groups received a capsule containing either placebo or 60,000 IU vitamin A, once only at baseline. Although the group administered the rehydrated mango showed an increase in plasma β-carotene concentrations, no significant differences were observed at 8 weeks between the mango, vitamin A, and placebo groups in terms of plasma β-carotene or retinol concentrations after adjusting for baseline characteristics (Drammeh et al. 2002).

Table 2 shows a summary of the data at 8 and 9 weeks reported in the above-mentioned studies conducted in Gambia and Indonesia, respectively. The Gambian children were slightly younger than those from Indonesia, which may account for the different baseline retinol concentrations. It should also be noted that plasma β-carotene concentrations were 3–4 times higher in the Gambian than in the Indonesian children; probably due to the presence of greater amounts of β-carotene in the Gambian diet compared with the Indonesian diet, which leads to greater availability of β-carotene for conversion to vitamin A during absorption. The amount of β-carotene given to Indonesian children was approximately double that given to the Gambian children, but on a body weight basis, the amount of supplement was probably similar for the two groups.

The Gambian children showed no response to any of the supplements at 8 weeks, even though children of that age should have a mean plasma retinol concentrations of ~1.0 μmol/L. Apparently-healthy preschool children in industrialized countries, for example in the UK (Thurnham et al. 2003), have plasma retinol concentrations of this order and these increase gradually to ~2.0 μmol/L through the teenage years (Ballew et al. 2001). This discrepancy may, however, be partly explained by the observation of evidence of inflammation in the Gambian children, in that almost 40% and 12% had elevated CRP concentration at baseline and 8 weeks, respectively.

Among the Indonesian children, more than 80% were infected with gut parasites, but no information was provided on any systemic effects of these infections. All three interventions produced significant increases in plasma retinol concentrations and, probably because of the low baseline carotene concentrations, significant increases in plasma β-carotene. The results clearly showed that the regular intake of the food supplement by the Indonesian children had a beneficial effect on their vitamin A status, and that the fresh fruit supplement was more efficacious than dark green leafy vegetables in increasing plasma β-carotene concentrations.

Despite the finding in the Gambian study of no apparent benefit of consuming a fat supplement in conjunction with the rehydrated mango, many other studies have shown that to optimize carotenoid absorption, dietary fat must be consumed during the same eating period as the carotenoids (Institute of Medicine 2000). For this reason, red palm oil is considered a good source of β-carotene and, in a small study of vitamin A-deficient school children in India, β-carotene from red palm oil was found to be as bioavailable as preformed vitamin A (Mahapatra and Manorama 1997). Furthermore, when the diet of pregnant women was supplemented with red palm oil for 8 weeks during the third trimester, the mean plasma retinol concentration of the women and their infants was significantly higher than was found when women received an equivalent amount of groundnut oil (Radhika et al. 2003). However, a 6 month intervention trial in Tanzania beginning in the third trimester to

TABLE 2

Effects of fruit or dark green leafy vegetable (DGLV) supplements on plasma β-carotene and retinol concentrations in Gambian and Indonesian children

Subjects	Intervention		μg RAE##	Plasma retinol μmol/L		Plasma β-carotene μmol/L	
	Administration/duration	supplement		Baseline	8–9 weeks	Baseline	8–9 weeks
Gambian* - 2–7 years (Drammeh et al. 2002)	once a day, 5 days/week for 8 weeks	75 g dried mango plus fat	150	0.64	0.63	0.52	0.53
		75 g dried mango	150	0.62	0.62	0.49	0.57
		Retinyl palmitate capsule	60,000	0.62	0.61	0.47	0.58
		Placebo capsule	zero	0.63	0.62	0.43	0.55
Indonesia**- Mainly anemic, 7–11 years (De Pee et al. 1998)	2 meals per day, 6 days/wk, for 9 weeks	DGLV + carrots	342	0.73	0.80#	0.14	0.28#
		Carotene-rich fruit	268	0.71	0.83#	0.15	0.67#
		Retinol-rich protein	534	0.69	0.92#	0.14	0.20#
		Low carotene and low retinol foods	22	0.70	0.70	0.14	0.17#

* 38% and 12% of the children had elevated CRP at baseline and 8 weeks, respectively
** Percent with positive stool parasites: 48–62% *Ascaris lumbricoides*; 78–82% *Tricuris trichuria*; 4–22% *Entamoeba histolytica*
# Significant increase compared with baseline P<0.05
1 μg retinol activity equivalent (RAE) is 1 μg all-*trans* retinol or 12 μg β-carotene

investigate the effect of red palm oil compared with sunflower oil on serum retinol concentrations showed no difference in maternal plasma retinol concentrations between the two groups, but a significant increase in concentrations of α- and β-carotene in maternal serum and in breast milk in the women receiving the red palm oil (Lietz et al. 2001). The similar effects of the two oil supplements on plasma retinol may have been due to there being no vitamin A deficiency in this population, as indicated by the high mean concentrations of plasma β-carotene at baseline in the two groups (0.52 to 0.63 μmol/L). The presence of high plasma β-carotene concentrations in Tanzanian women is not an isolated example, as discussed in the section on cross-sectional and case-control studies in connection with the work of Mulokozi et al. (2003)

One interesting study that confirmed the importance of fat in the diet and highlighted the adverse effects of intestinal parasites on vitamin A status is that of Jalal et al. (1998) (Table 3, p. 10). The authors showed that the addition of β-carotene rich foods to the diets of preschool children in Indonesia (without additional fat and antihelminthics) improved vitamin A status; however, they also reported that vitamin A status improved almost as well when only the fat and antihelminthic drug were given, without the additional β-carotene. Given that a separate analysis of the data showed that children with heavy *A. lumbricoides* infections had a mean serum retinol concentration that was 15% lower than that of children with light infections, the effect on retinol of fat alone in conjunction with the antihelminthic drug may have been due to a partial removal of inflammation. In contrast, supplying extra β-carotene plus fat and antihelminthic treatment increased the plasma retinol concentration by more than 50%, to levels that were almost normal for children of this age. It should be noted that the β-carotene was supplied in the form of sweet potato, and the authors suggest that β-carotene from this source may be more bioavailable than that in green leafy vegetables. This difference in β-carotene bioavailability may explain why this study was more effective in raising plasma retinol concentrations than the earlier study of de Pee et al. (1995) discussed above, which used green leafy vegetables. These considerations highlight the need to control as many factors as possible when determining the bioefficacy values for β-carotene in

TABLE 3

INFLUENCE OF DIETARY FAT AND ANTIHELMINTHIC TREATMENT ON THE BIOEFFICACY OF β-CAROTENE+

Meal	µg RAE	Subjects	Mean serum retinol µmol/L		
			Baseline	Post treatment	Change
Basic meal	100 (mainly vegetables)	3–6 year olds, both sexes	0.66	0.70	0.035
Basic meal, fat*, and antihelminthic**	100		0.64	0.81	0.17
Basic meal plus β-carotene#	475		0.57	0.79	0.22
Basic meal, fat, β-carotene plus drug	475		0.59	0.94	0.345

+ modified from Jalal et al. 1998
* 15 g per day as coconut oil or coconut milk
** levamisole given about 1 week before commencement of food supplement study
# 375 RAE, approximately 80% of which was from sweet potato

plant foods. Some researchers have argued that it is more important to study the "functional" bioefficacy of foods to improve vitamin A status, that is, to obtain results under environmental conditions as they exist and not as we would perhaps like them to be (van Lieshout et al. 2003). We maintain, however, that it is more important to determine the true bioefficacy; thus the removal of any infection (e.g., by antihelminthics), addition of fat, and optimized food preparation be done where this might affect carotene absorption, and any residual inflammation should be quantified (Thurnham et al. 2003) because it could conceal changes in plasma retinol concentrations.

Isotopic tracer techniques are increasingly being used to study the bioavailability and bioefficacy of dietary carotenoids, especially β-carotene (van Lieshout et al. 2003). These techniques have the advantage that they enable quantitation of body stores of retinol at baseline and following an intervention. For example, Haskell et al. (2004) recently estimated total body vitamin A stores in 14 Bangladeshi men using the deuterated retinol dilution technique before and after 60 day supplementation with white vegetables (0 µg RAE) or 750 µg RE of sweet potato, Indian spinach, retinyl palmitate, or β-carotene (RE = 1 µg retinol or 6 µg β-carotene). The mean changes in the retinyl palmitate group were compared with the mean changes in the other groups to estimate the relative equivalence of these vitamin A sources. Vitamin A equivalency factors (β-carotene:retinol, wt:wt) were estimated as ~13:1 for sweet potato, 10:1 for Indian spinach, and 6:1 for synthetic β-carotene. It is noteworthy that the β-carotene:retinol equivalencies for both sweet potato and Indian spinach were close to 12:1, the figure provisionally proposed by the Institute of Medicine (2001). Both vegetable foods were pureed and/or sautéed in oil to promote optimal bioavailability. The subjects showed no clinical evidence of intestinal malabsorption, their serum albumin and CRP were >35 g/L and <10 mg/L, respectively, and all were treated with Albendazole during the week prior to intervention. The authors suggest that their study showed that the percentage change in vitamin A pool size was negatively related to the initial vitamin A pool size. That is, individuals with poorer vitamin A status were more responsive to treatment, consistent with the findings of Parvin and Sivakumar (2000). Also noteworthy was that the β-carotene:retinol equivalency for synthetic β-carotene was 6:1, which is higher than the figure proposed by the Institute of Medicine (2001). The authors suggest that this discrepancy may have arisen because they fed their daily doses of β-carotene with a meal that contained ~29 g dietary fiber that may have reduced absorption.

The above study attempted to optimize the factors that might influence absorption of β-carotene from food. However, the observation of a greater vitamin A pool-size response in individuals with poorer vitamin A status at baseline indicates the difficulty in establishing single equivalence factors for foods.

Nevertheless, further studies in different communities are needed to measure the impact of provitamin A-containing foods on vitamin A status. The few studies published to date have been used to define the conversion factors; however, additional studies on fruits, vegetables, tubers, and even cereals should be conducted to determine the factors influencing the absorption and bioconversion of provitamin A carotenoids. Furthermore, additional studies are needed to quantify how dietary factors such as fat, fiber, and possibly other nutrient deficiencies influence the bioefficacy of retinol formation from provitamin A carotenoids.

Meeting vitamin A requirements by consumption of plant foods

Provitamin A carotenoids in plant foods, when consumed with sufficient fat, can provide enough vitamin A to support a healthy life. In developing countries, however, vegetables and fruits are usually consumed in insufficient amounts as part of a low fat diet. In addition, parasitic and other infections increase the vitamin A requirements, and children and pregnant and lactating women are often vitamin A-deficient. Based on available data, Miller et al. (2002) calculated that the diets of children in developing countries without vitamin A supplementation do not meet vitamin A requirements, due to the low concentrations of vitamin A in breast milk of vitamin A-deficient mothers, inadequate dietary intake of vitamin A during and after weaning, and the high prevalence of childhood illnesses. They proposed that children in these countries need to consume 10 times their current intake of fruits and vegetables if they are to overcome the vitamin A deficit by eating fruits and vegetables alone. It needs to be stressed, however, that this is a theoretical estimate based on models that contain many assumptions.

Increasing the β-carotene content in plant foods such as vegetables, tubers, and fruits through plant breeding techniques may be a sustainable approach to improving vitamin A status in developing countries as it does not require a change of eating habits, provided the food is already an important dietary component. One approach to substantially increasing bioavailable provitamin A carotenoid intake is to increase the density of carotenoids in foods, as well as their absorption and bioconversion. In this respect, fruits and tubers may be preferable to dark green leafy vegetables. Bioavailability of nutrients in mineral- and vitamin-dense staple crops is a major determinant of whether they improve the nutritional status of undernourished populations (Khush 2002).

One recent research area aimed at developing a staple food rich in β-carotene has been the genetic modification of rice, which is normally devoid of vitamin A and carotenoids. This crop was chosen because vitamin A deficiency is particularly prevalent in poor, rice consuming populations, who often cannot afford to buy animal source foods or sufficient fruits and vegetables. Ye et al. (2000) made an important breakthrough by using genetic engineering to express a series of enzymes from daffodil and a microorganism in rice endosperm so that β-carotene was biosynthesized in the new Golden Rice. More recently, Paine et al. (2005) developed Golden Rice 2, which contains 37 mg/g carotenoid, of which 31 mg/g is β-carotene. However, although the quantity of β-carotene is high, its bioavailability is unknown. Taking into account the low vitamin A concentration in breast milk in developing countries, Miller et al. (2002) estimated that a child during the second year of life needs to consume 160–200 μg RAE/day. If the conversion factor of 1:12 for μg of RAE:μg of β-carotene (Institute of Medicine 2001) is used, the extra amount of β-carotene needed would be 1.92–2.4 mg/day. If the child is not receiving any breast milk, then to meet the RDA of 300 μg RAE requires 3.6 μg β-carotene/day. We calculated the amount of β-carotene that would be obtained by Thai infants (1–2 years) if they ate Golden Rice at intake levels similar to that reported by Thurnham et al. (1971). As in that study, we assumed infants are still receiving breast milk and their daily rice consumption is 160 g (standard deviation 29 g). This equates to ~67 g uncooked rice, which would contain 2.077 μg β-carotene and hence would provide approximately 58% of the RDA. This is a promising figure; however,

for genetically modified rice to come into widespread use, its bioavailability and acceptability need to be established. Acceptable bioavailability may be attainable by genetic means or food preparation methods, but achieving public acceptance may require both political and social changes as food preferences are deeply engrained in public beliefs.

In conclusion, long term prevention of vitamin A deficiency in developing countries can be best achieved by combining a variety of strategies, including improved general health status, increased consumption of foods rich in vitamin A and/or provitamin A carotenoids, food fortification with vitamin A, and by increasing the native content of provitamin A carotenoids in fruits, vegetables, tubers, and cereals through plant breeding and biotechnology.

Link between vitamin A deficiency and iron status

Low intake of vitamin A from animal source foods or low intake and/or bioavailability of provitamin A carotenoids from plant foods may negatively impact on iron status through the negative influence of a low vitamin A status on iron metabolism. In such a situation, therefore, increasing the intake of vitamin A or bioavailable provitamin A carotenoids may increase iron utilization, thereby improving iron status. The existence of a link between vitamin A deficiency and anemia has been known for many years. However, the stage of iron metabolism at which vitamin A exerts its critical effect remains obscure. Several mechanisms have been proposed (Roodenburg et al. 2000) and, in tropical countries, the high prevalence of infectious diseases may also play a role as vitamin A deficiency can decrease immune function, increase infections and, due to a modulation of hematopoiesis (Thurnham 1993), increase the anemia of infection (Means 2000).

Early animal studies first indicated a link between vitamin A and iron metabolism. Vitamin A deficiency in rats (Sure et al. 1929) and in dogs (Crimm and Short 1937) resulted in anemia, and the provision of vitamin A to vitamin A deficient rats resulted in a rapid rise in hemoglobin (Koessler et al. 1926). Roodenburg et al. (1994) confirmed that vitamin A deficiency in rats produces a mild anemia as the first change in iron metabolism.

The first report linking vitamin A to anemia in humans was that of Blackfan and Wolbach (1933), who observed anemia, together with hemosiderosis of the spleen and liver, in vitamin A deficient infants. They further found that repletion of vitamin A was followed by regeneration of the bone marrow, disappearance of hemosiderin from the spleen and liver, and increased erythroblastic activity. Already in 1940, Wagner (1940) proposed that vitamin A deficiency impaired hematopoiesis, based on the observation that adult subjects developed low blood hemoglobin levels and low packed cell volumes after consuming a low vitamin A diet for 6 months. This was confirmed by Hodges et al. (1978), who additionally showed that providing high iron supplements to individuals with concurrent iron and vitamin A deficiency did not increase hemoglobin levels.

In the study of Hodges et al. (1978), 8 middle-aged men were fed a combination of 3 different vitamin A deficient diets together with mineral and vitamin supplements for 360–770 days. The intake of all nutrients, except vitamin A, was judged adequate. However, despite a daily intake of 18–19 mg iron, the men developed mild anemia after about 6 months. As plasma retinol levels fell from what was described as plentiful (>1.05 μmol/L or 30 μg/dL) to adequate (20–30 μg/dL) and on to low (<0.7 μmol/L or 20 μg/dL), the mean hemoglobin values fell from 156 g/L to 129 g/L and on to 119 g/L. The anemia was not responsive to iron therapy until the subjects were replete with vitamin A, provided as a β-carotene supplement (360 μg retinol/day as β-carotene for 193 days).

Cross-sectional studies

Despite the influence of other nutritional factors and infectious diseases on both vitamin A and iron status, many cross-sectional studies in developing countries have reported a positive correlation between serum retinol and hemoglobin concentration. This correlation can arise from common risk factors or shared metabolic pathways. The correlation becomes stronger with

TABLE 4

CORRELATIONS BETWEEN BLOOD HEMOGLOBIN AND PLASMA RETINOL CONCENTRATIONS IN CROSS-SECTIONAL STUDIES

Country	Subjects	Mean plasma retinol µmol /L	Mean hemoglobin g/L	Correlation			Reference
				r	n	p	
Pooled data from eight countries	Female 15–45 years, nonpregnant & nonlactating	1.06	130	0.77	8	<0.05	Hodges et al. 1978
Central America	School children 1–4 years	~0.84#	~120#	0.12	500	NS	Mejia et al. 1977
	School children 5–8 years	~0.84#	~130#	0.21	536	<0.05	
	School children 9–12 years	~0.98#	~134#	0.18	477	<0.1	
India	School children 4–12 years	0.76	111	0.52	110	<0.001	Mohanram et al. 1977
Bangladesh	School children 5–12 years	~1.4##	~138##	0.31	242	<0.001	Ahmed et al. 1993
Malawi	Adolescent, nonpregnant girls 10–19 years	0.92	99	0.16	118	=0.08	Fazio-Tirrozzo et al. 1998
			Mean serum iron µmol/L				
Central America	School children 1–4 years	~0.88#	~14.2#	0.21	412	<0.05	Mejia et al. 1977
	School children 4–8 years	~0.79#	~13.4#	0.18	492	<0.1	
	School children 9–12 years	~0.98#	~14.2#	0.21	447	<0.05	
Austria	Elderly adults	1.66	20.31	0.56	39	<0.001	Wenger et al. 1979

Values visually estimated from graphs
Values estimated from subgroups

lower vitamin A status (Fishman et al. 2000). Hodges et al. (1978) found a high positive correlation ($r = 0.77$, $p<0.0001$) in an analysis of pooled data from surveys in Vietnam, Chile, Brazil, Uruguay, Ecuador, Venezuela, Guatemala, and Ethiopia. Likewise, as shown in Table 4, positive correlations between serum retinol and hemoglobin were found in school children in Central America ($r = 0.21$, $p<0.05$) (Mejia et al. 1977), India ($r = 0.52$, $p<0.001$) (Mohanram et al. 1977), and Bangladesh ($r = 0.31$, $p<0.001$) (Ahmed et al. 1993), in adolescent girls from Malawi ($r = 0.16$, $p = 0.08$) (Fazio-Tirrozzo et al. 1998), and in older adults in Vienna ($r = 0.56$, $p<0.001$) (Wenger et al. 1979). In a recent study in Indonesia, infants and mothers with low vitamin A status (serum retinol <0.7 µmol/L) had a 2.4 fold increased risk for IDA (Dijkhuizen et al. 2001). Similarly in Tanzania, pregnant women with hemoglobin levels below 90 g/L were found to be 2.2 fold more likely to have low serum retinol concentrations, and anemia in these women was also found to be associated with elevated serum CRP concentrations (Hinderaker et al. 2002).

Intervention studies

Many intervention studies have shown that vitamin A supplements, or foods fortified with vitamin A, improve blood hemoglobin concentrations in children and in pregnant or lactating women (Semba and Bloem 2002). In addition, studies have shown that dual supplementation with iron and vitamin A has a greater impact on hemoglobin concentrations than iron alone in both children (Mwanri et al. 2000) and pregnant women (Suharno et al. 1993) (Table 5, p. 14). However, other studies have shown no additional significant effect of vitamin A on hemoglobin concentrations (Villamor et al. 2000). The lack of effect observed in the last two studies may have been due to the study populations having satisfactory vitamin A status. The study by Villamor et al. (2000) was a randomized, placebo-controlled trial in 6–60 month old Tanzanian children who were admitted with pneumonia. They were given four doses of 30 or 60 µg RAE (depending on age), two in hospital and two more at 4 and 8 months, but no effect on hemoglobin was observed at 1 year. No information was given on the vitamin A status of the children, but other studies in Tanzania (Lietz et al. 2001;

TABLE 5

Intervention studies showing effects of vitamin A and/or iron supplementation on blood hemoglobin concentration

Country	Supplement	Vehicle/treatment	Duration of study	Subjects	Aspects concerning morbidity	Hemoglobin			Reference
						Baseline g/L	Increase g/L	P<**	
Guatemala	3–5 drops of control solution	Dosing as for iron	2 months	Anemic children 1–8 year old randomized by center and age ±3 years as far as possible	Morbidity monitored during study but no mention of any pre-treatment. High incidence of morbidity associated with Fe suppl groups	104	3.2	NS	Mejia and Chew 1988
	3 mg RAE/day	2 drops AROVIT orally post breakfast				103	9.3	0.05	
	3 mg Fe/kg/day	Fer-In-Sol (ferrous sulfate) mid-morning and mid-afternoon				105	13.8#	0.001	
	3 mg RAE/day + 3 mg Fe/kg/day	As above for vitamin A and iron				106	14.2#	0.001	
Indonesia	Placebo	Separate capsules of iron, vitamin A, and placebo	8 weeks	Pregnant women with hemoglobin between 80 and 109 g/L	No mention of deworming in report	103	2.0	NS	Suharno et al. 1993
	2.4 mg RAE/day					103	3.7	0.001	
	60 mg Fe/day					103	7.7	0.001	
	2.4 mg RAE/day + 60 mg Fe/day					103	12.8##	0.001	
Bangladesh	60 mg Fe /day	Fe as ferrous sulfate; vitamin A given once; Zn as zinc gluconate	60 days; compliance observed twice weekly	Nonpregnant anemic (<100 g/L) women 15–45 years old	Women with obvious acute or chronic disease and inflammatory conditions were excluded	92.9	13.4	0.001	Kolsteren et al. 1999
	60 mg RAE +60 mg Fe/day					89.5	15.9	0.001	
	60 mg RAE + 60 mg Fe/day + 15 mg Zn/day					88.5	17.9###	0.001	
Tanzania	Placebo	Corn gruel given 3 days/week	3 months	Anemic school-children 9–12 years old	Dewormed 2 weeks before intervention	104	3.5	0.001	Mwanri et al. 2000
	1.5 mg* RAE					104	13.5	0.0001	
	40 mg* iron					106	17.5	0.0001	
	1.5 mg RAE + 40 mg* iron					107	22.1##	0.0001	

* Dose per day is equivalent to 0.65 mg RAE for vitamin A and 17 mg iron

** Final value significantly different from baseline

Although the change in hemoglobin was not significantly different for the groups treated with iron alone and iron plus vitamin A, the mean change in serum iron in those receiving vitamin A plus iron was much greater than in those receiving iron or vitamin A alone

Effect of vitamin A plus iron stronger than that of iron or vitamin A alone

Effect of vitamin A, iron, and zinc significantly different from that of iron alone

Mulokozi et al. 2003) suggest that mothers and therefore children have satisfactory vitamin A intake. In the case of the study by Kolsteren et al. (1999) in Bangladesh, the authors comment that serum retinol values were well within the normal range and only 6% had retinol concentrations <0.7 μmol /L (Table 5).

Intervention studies in which vitamin A alone improved hematological status include that of Muhilal et al. (1988), who demonstrated that vitamin A-fortified monosodium glutamate not only improved the vitamin A status of preschool children in Indonesia but also increased hemoglobin by 10 g/L over 5 months. Similarly, supplementation with vitamin A alone was found to increase blood hemoglobin levels in children in Thailand (Bloem et al. 1989; Bloem et al. 1990), Indonesia (Semba et al. 1992) and Tanzania (Mwanri et al. 2000), and in pregnant women in Indonesia (Suharno et al. 1993) (Table 6, p. 15). Several studies have additionally explored combined supplementation with vitamin A and iron (Table 5). In another study in Indonesia, 251 anemic pregnant women with marginal

TABLE 6

Intervention studies showing effects of vitamin A supplementation on blood hemoglobin concentrations

Country	Supplement	Vehicle	Duration of study	Subjects	Hemoglobin			Reference
					Baseline g/L	Increase g/L	p	
Indonesia	Vitamin A	Mono-sodium glutamate	11 months	1–6 years old	113 (16)	10		Muhilal et al. 1988
Thailand	Single dose 60,000 μg RAE	Capsule	2 months	166 children 1-8 years old, Hb<7.5 mmol (120 g/L#)	111.4	4.2	<0.05*	Bloem et al. 1989
			4 months		111.4	3.2	<0.05*	
Thailand	Single dose 60,000 μg RAE	Capsule	2 weeks	134 children 3–9 years old	115.4	2.2	<0.05	Bloem et al. 1990
Indonesia**	60,000 μg RE##	Capsule	5 weeks	3–6 years old with Hb<110 g/L at baseline	102 (n=7)	21	<0.001	Semba et al. 1992
	Placebo				101 (n=14)	14	<0.001	

* Treatment effects not significantly different from controls at either 2 or 4 months, probably because mangoes became available in the 2 months following the baseline survey
** Although there appeared to be an increase in hemoglobin in the total sample of 118 clinically normal children and 118 with mild xerophthalmia following vitamin A supplementation, the effect was not significant as there was no difference between the effects of vitamin and placebo
# mmol/L converted to g/L (× 16.07)
1 retinol equivalent (RE) is 1 μg RAE

vitamin A status were randomly allocated to receive placebo, vitamin A (2.4 mg RE/day), iron (60 mg/day), and iron plus vitamin A (60 mg Fe/day, 2.4 mg RE/day) for 8 weeks (Suharno et al. 1993). After supplementation, the percentage of women who were no longer anemic was 16%, 35%, 68%, and 97%, respectively, in the 4 groups. In a similar 3 month supplementation study of anemic school children in Tanzania, 1.5 mg RE/day increased hemoglobin by 13.5 g/L, 40 mg iron/day increased hemoglobin by 17.5 g/L, and the combined treatment increased hemoglobin by 22.1 g/L, compared with the placebo effect of 3.6 g/L (Mwanri et al. 2000). However, other studies found no additional improvement in hemoglobin levels when vitamin A plus iron was administered compared with iron alone (Mejia and Chew 1988).

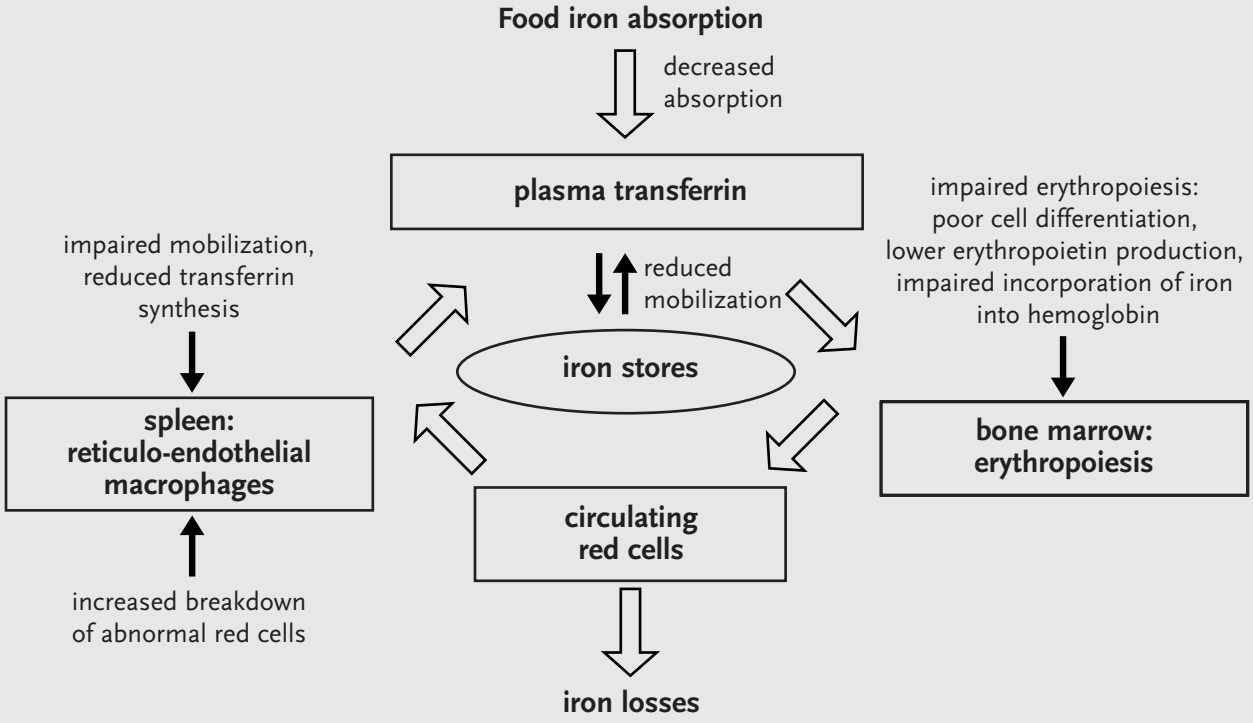
The studies described above suggest that both vitamin A and iron are required for normal red cell production, although the exact nature of the interaction is not known. It would appear that vitamin A may be needed for erythropoiesis, including the incorporation of iron into hemoglobin, and/or for mobilization of iron from the spleen or liver stores. Alternatively, the positive influence of vitamin A on the immune system may decrease the anemia of infection. Because other nutritional and disease factors can also influence erythropoiesis, it is not possible to generalize on the extent of improvement in IDA when both iron and vitamin A are administered in the same fortification or biofortification package. However, where vitamin A status is marginal and iron deficiency or anemia is common, dual fortification, biofortification, or supplementation is recommended. If other micronutrients are also lacking in the diet, these may also need to be provided to obtain the maximum impact of iron fortification or biofortification on iron status.

Possible interactions between vitamin A and iron metabolism

Iron metabolism can be described as a closed loop in which the primary processes are the formation and destruction of red blood cells. Small amounts of iron enter this loop via the absorption of dietary iron and, in balance, an equivalent amount of iron exits the loop as losses from blood and tissues (Figure 1, p. 16). Vitamin A has been proposed to influence iron metabolism either via its effect on erythropoiesis, with vitamin A deficiency leading to decreased erythropoiesis with less iron incorporated into red blood cells (Roodenburg et al. 2000), or indirectly by its beneficial effects on immune function leading to a decrease in the anemia of infection (Thurnham 1993). In addition, as infection is reported to block iron absorption (Beresford et al. 1971),

FIGURE 1

Possible influences of vitamin A deficiency on iron metabolism*



* Influence of infection and inflammatory disorders are more common in vitamin A deficiency as a result of a decreased immune defense and may additionally impair iron absorption, its mobilization from stores, and erythropoiesis

the promotion of immune function by vitamin A may remove this blockade of iron absorption by reducing inflammation. However, testing these theories and confirming the effect of vitamin A on iron absorption has proved difficult, and the exact mechanism by which vitamin A interacts with iron metabolism remains obscure.

In rats, vitamin A deficiency reduced the incorporation of radioactive iron into erythrocytes by almost 50% (Mejia et al. 1979a), altered red blood cell morphology (Mejia et al. 1979b), produced mild anemia (Sijtsma et al. 1993; Roodenburg et al. 1994), and lowered plasma total iron binding capacity and percent transferrin saturation (Mejia and Arroyave, 1983; Roodenburg et al. 1994; Roodenburg et al. 1996), but not circulating transferrin concentration (Mejia and Arroyave 1983). Vitamin A deficiency also caused an accumulation of iron in the liver (Mejia et al. 1979a; Staab et al. 1984), spleen (Mejia et al. 1979a; Roodenburg et al. 1994; Roodenburg et al. 1996), and bone (Roodenburg et al. 1994; Roodenburg et al. 1996). Furthermore, vitamin A deficiency in rats appeared to increase iron absorption from the gut (Mejia et al. 1979a; Sijtsma et al. 1993; Roodenburg et al. 1994).

Based on these findings, Roodenburg et al. (1996) hypothesized that vitamin A deficiency impairs erythropoiesis such that mild anemia with malformed cells develops. The resulting abnormal erythrocytes would be broken down by the macrophages of the reticuloendothelial system at an increased rate, which would explain, at least in part, the accumulation of iron in the spleen. In a subsequent study, however, Roodenburg et al. (2000) could find no evidence in rats that vitamin A deficiency affects erythropoiesis, leading them to speculate that iron accumulation in the spleen may be related to reduced iron transport due to an inhibition of transferrin synthesis, a hypothesis that is inconsistent with an earlier report of no decrease in circulating

transferrin concentrations in vitamin A deficient rats (Mejia and Arroyave 1983). Nevertheless, the possibility remains that vitamin A is involved in the release of iron from spleen or liver stores, or perhaps directly in the incorporation of iron into hemoglobin. Evidence suggesting an influence of vitamin A on the mobilization of liver and spleen iron comes from the study of van Stuijvenberg et al. (1997). Specifically, they found that children receiving iron fortified soup showed a greater increase in serum iron levels and transferrin saturation when their plasma retinol levels were >40 μg/dL (1.4 μmol/L) compared with <20 μg/dL (0.7 μmol/L).

In vitro studies have indicated that retinoids influence erythropoiesis via a complex mechanism that depends on the stage of erythrocyte development (Perrin et al. 1997), and may involve a direct effect on erythropoietin formation (Semba et al. 2001). The process of red cell formation in the bone marrow involves the differentiation of stem cells through a series of cell types, including erythroid burst-forming units, erythroid colony forming units, proerythroblasts, orthochromic erythroblasts, reticulocytes, and mature erythrocytes. Hemoglobin synthesis, including the incorporation of iron into protoporphyrin, occurs during the differentiation of the erythroid colony forming units (Bondurant et al. 1985) and continues until the reticulocyte matures into a mature erythrocyte (Izak et al. 1971). Erythropoiesis is under the control of erythropoietin, a glycoprotein produced in the renal cortical cells, which causes the erythroid progenitor cells to differentiate into proerythroblasts (Erslev 1991).

Retinoic acid was shown in vitro to stimulate human erythroid burst-forming unit colony formation, suggesting that retinoids are involved in erythropoiesis (Douer and Koeffler 1982). Subsequent studies have confirmed that retinoic acid stimulates early erythroid colonies in synergy with erythropoietin (Correa and Axelrad 1992), but that the effects are complex and depend on culture conditions, cell maturation stage, and the cytokines used for stimulation (Semba and Bloem 2002). The erythropoietin gene appears to contain a retinoic acid responsive element, and when vitamin A deficient rats were fed retinoic acid, serum erythropoietin concentrations increased (Okano et al. 1994).

Similarly in isolated, perfused rat kidneys, vitamin A increased renal erythropoietin synthesis (Neumcke et al. 1999). However, in a clinical trial in pregnant women in Malawi, Semba et al. (2001) found that vitamin A supplementation had no effect on erythropoietin production.

In addition to the effects of vitamin A deficiency on iron metabolism, it also appears that iron deficiency can impair vitamin A metabolism by decreasing vitamin A mobilization from liver stores and perhaps also by decreasing retinol absorption (Jang et al. 2000). Iron (and zinc) supplementation have been reported to improve indicators of vitamin A status in Mexican school children (Munoz et al. 2000).

Infection

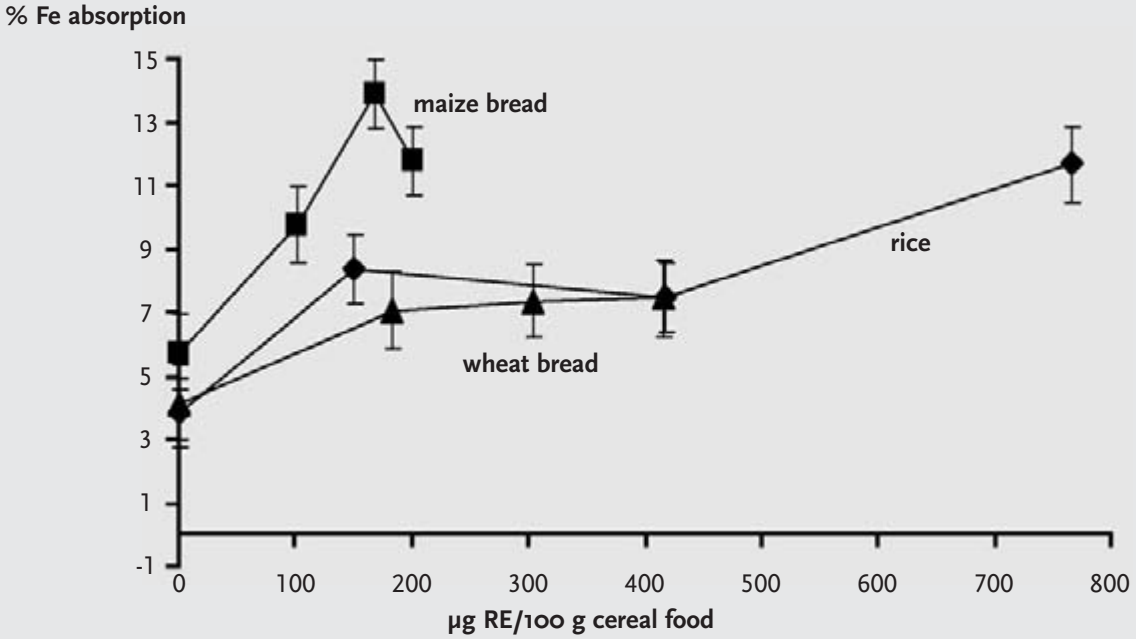
Although it seems reasonable to speculate that vitamin A enhances immunity and reduces infection and thus the anemia of infection, there are few data directly supporting these assertions (Semba and Bloem 2002). Anemia of infection occurs despite adequate reticuloendothelial iron and is observed for example in children where there is high exposure to chronic infection (Jansson et al. 1986), or subjects with tuberculosis (Morris et al. 1989) or human immunodeficiency virus (Semba and Gray 2001). Inflammatory cytokines such as interferon gamma, interleukin 1, and tumor necrosis factor appear to interfere with erythropoiesis (Murphy et al. 1988) and to increase ferritin production (Feelders et al. 1998).

Absorption

Studies investigating the influence of vitamin A on iron absorption in human subjects have generated contradictory results. Although the possibility that these discrepancies arise from methodological differences has not been completely ruled out, it now seems plausible that the contradictions in the results are caused by the effect of vitamin A on iron absorption depending on the nutritional or health status of the subject. In Venezuela, a series of studies with radioiron isotopes showed that iron absorption by poor Venezuelan subjects fed iron-fortified wheat bread, maize bread, and rice meals was increased when the subjects received supplemental vitamin A or β-carotene

FIGURE 2

Influence of vitamin A on iron absorption[a] from maize bread, wheat bread and rice meals[b] (Garcia-Casal et al., 1998)



[a] The iron absorption value includes iron that is absorbed from the gut and incorporated into hemoglobin

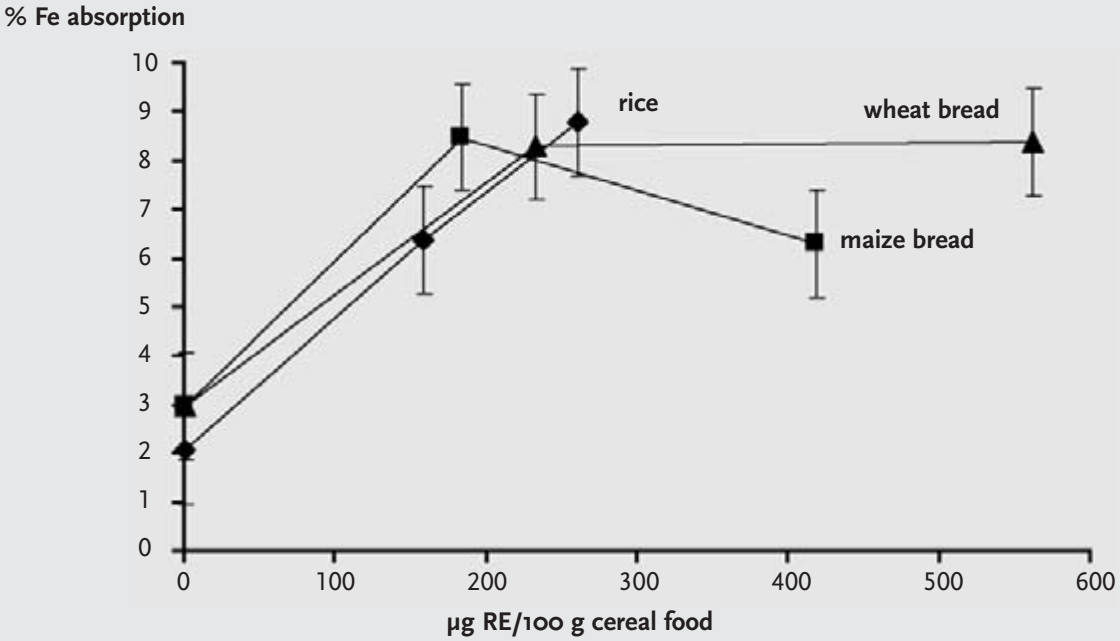
[b] Maize flour and wheat flour were both fortified with ferrous fumarate (5 mg Fe and 2 mg Fe respectively) and fed as a bread with margarine and cheese. Rice was unfortified and fed with margarine

(Layrisse et al. 1997; Garcia-Casal et al. 1998) (Figure 2). In the first of these studies (Layrisse et al. 1997), meals were consumed with coffee or tea, and it was proposed that vitamin A blocks the polyphenol and phytic acid-induced inhibition of iron absorption. The second study (Garcia-Casal et al. 1998), which investigated both β-carotene and vitamin A, demonstrated an enhancement of iron absorption from cereal foods in the absence of polyphenol-containing beverages. However, in a study of Swiss and Swedish students using a similar radioiron methodology and a stable isotope technique, Walczyk et al. (2003) found no influence of vitamin A (3.5 µmol) on iron absorption from iron fortified maize bread fed with and without coffee. To further complicate the issue, it was recently reported that vitamin A and iron deficient children in the Côte d'Ivoire fed a maize gruel showed inhibited iron absorption (35%) when the gruel was supplemented with 3.5 µmol vitamin A (Davidsson et al. 2003). Later, the same children were supplemented with a high dose of vitamin A (210 µmol), but 3 weeks afterwards iron absorption still remained at the lower level. The methods used to estimate iron absorption in all studies were similar and based on erythrocyte incorporation of either stable or radioactive isotopes. This methodology quantifies iron that is first absorbed and then incorporated into hemoglobin. Any effect of vitamin A could be on the absorption stage, or on the subsequent incorporation of iron into hemoglobin, or on both the absorption and utilization.

The inconsistencies in the results of the studies on the influence of vitamin A on iron absorption are difficult to explain. The differences in the methodologies used seem too small to account for the discrepancies in the results. It seems more probable that differences in the nutritional status of the study subjects or their subclinical disease state at the time of the absorption study gave rise to their different responses to dietary vitamin A. A detailed nutritional status evaluation of

FIGURE 3

Influence of β-carotene on iron absorption[a] from maize bread, wheat bread and rice meals[b] (Garcia-Casal et al., 1998)



[a] The iron absorption value includes iron that is absorbed from the gut and incorporated into hemoglobin

[b] Maize flour and wheat flour were both fortified with ferrous fumarate (5 mg Fe and 2 mg Fe respectively) and fed as a bread with margarine and cheese. Rice was unfortified and fed with margarine

the study subjects from Venezuela and the Côte d'Ivoire was not made nor was the extent of in vivo inflammation assessed in any of the studies therefore the true extent of vitamin A and iron deficiency is uncertain. Results from previous studies in the Côte d'Ivoire, however, suggest that the study subjects in that country were exposed to chronic infections, malaria, or intestinal parasites (Asobayire et al. 2001) and therefore both iron and vitamin A status could have been influenced by sub-clinical inflammation. In addition, erythropoiesis, including the incorporation of iron into hemoglobin, can be influenced by factors other than iron and vitamin A nutrition. For example, riboflavin (Powers 1995), folic acid, vitamin B12, and vitamin B6 status (Fishman et al. 2000) can all influence red blood cell formation, as can chronic infection (Jansson et al. 1986).

Taken together, the results from Venezuela, Côte d'Ivoire, and Europe highlight the complexity of the interaction of vitamin A status, dietary vitamin A, β-carotene, and iron bioavailability and emphasize the difficulty in extrapolating results from an industrialized to a developing country, or even from one developing country to another where different diets and lifestyles exist. In populations whose diets are lacking in both iron and vitamin A, it is recommended that iron be combined with vitamin A or β-carotene in iron fortification or biofortification programs; however, further studies are needed to elucidate the mechanism of the interaction between vitamin A and iron. As a first step toward resolving the apparently contradictory findings of previous studies, the influence of vitamin A (or β-carotene) on incorporation of iron into the hemoglobin of vitamin A replete and deficient subjects needs to be studied, and the influence of β-carotene and vitamin A on iron absorption needs to be measured using stable isotope methods in well characterized subjects in a developing and in an industrialized country. Moreover, in all future studies, vitamin A status should be quantified using the deuterated

retinol dilution method (Haskell et al. 2004) rather than the proxy indicators of plasma retinol or carotenoid concentrations, although the latter are a useful adjunct to assist interpretation. Furthermore, subclinical inflammation should be quantified using the serum acute phase proteins, CRP and AGP (Thurnham et al. 2003), because although inflammation status does not affect measurements of retinol stores (using the deuterated retinol method), it may influence the absorption of iron and erythropoiesis.

Link between vitamin A deficiency and zinc status

Although animal studies have shown a clear interaction between zinc status and vitamin A metabolism and vice versa, studies in humans have given inconsistent results and have provided no clear evidence that this interaction has any public health significance (Christian and West 1998). However, in view of the current difficulties in correctly measuring both vitamin A and zinc status, it is premature to assert that there are no benefits to be gained from dual fortification or biofortification of foods.

Animal studies

Animal studies have indicated that zinc status influences several aspects of vitamin A metabolism, including absorption, transport, and utilization (Christian and West 1998; Lönnerdal 1998). The mechanism mainly postulated to explain a potential dependence of vitamin A on zinc is related to the regulatory role of zinc on vitamin A transport mediated through protein synthesis (Mejia 1986). In zinc deficiency, production of retinol-binding protein can be reduced, resulting in secondary vitamin A deficiency that is reflected in low serum vitamin A concentrations (Smith et al. 1974). The other role of zinc may be in the visual cycle. Although zinc deficiency was previously thought to impair the activity of zinc dependent 11-cis retinol dehydrogenase, the enzyme required for conversion of all-trans retinal to 11-cis retinal in the retina (Huber and Gershoff 1975), it is now known that the enzyme is zinc independent (Dorea and Olson 1986; Duester 1996; Christian and West 1998). However, numerous other enzymes important in the visual cycle may be zinc dependent. Zinc deficiency has been shown in a number of species to result in a variety of gross, ultrastructural and electrophysiologic ocular manifestations (Grahn et al. 2001).

Zinc may also affect absorption of vitamin A. For example, zinc deficiency in rats lowers the intestinal absorption of fat and fat-soluble vitamins including retinol (Ahn and Koo 1995a; Ahn and Koo 1995b; Kim et al. 1998). The reduced intestinal absorption of retinol was attributed to a decrease in lymphatic phospholipid output resulting from impaired biliary secretion into the intestinal lumen. In addition, zinc deficiency significantly reduces lymphatic output of retinol during intraduodenal infusion of β-carotene in rats (Noh and Koo 2003). The mechanism underlying the effect of zinc on β-carotene absorption is unknown. Moreover, the extent to which zinc deficiency in humans affects absorption of β-carotene and its conversion to retinol is unknown, as the conversion of β-carotene to retinol varies substantially among animal species (Lee et al. 1999).

An interaction is also possible in the other direction, and several animal studies have reported negative effects of vitamin A deficiency on zinc metabolism. For example, vitamin A deficient rats show altered tissue zinc concentrations (Rahman et al. 1995; Rahman et al. 1999) and significantly lower serum zinc levels compared with controls of normal vitamin A status (Kanazawa et al. 2002). In addition, chicks fed a vitamin A-deficient diet showed lower plasma zinc concentration and higher hepatic zinc concentration compared with vitamin A-replete controls (Sklan et al. 1987). A decrease in zinc absorption of 40% in the small intestine and of 57% in the ileum was found in severely vitamin A-deficient chicks and a vitamin A-dependent zinc-binding protein was identified in the ileal mucosa (Berzin and Bauman 1987). In that study, however, the chicks manifested severe hypovitaminosis A, weighed 40% less than the controls, and developed secondary zinc deficiency. A dramatic decrease in zinc absorption is likely to occur in cases of such severe vitamin A deficiency, but the effects on zinc absorption of milder vitamin A deficiency remain unknown (Christian and West 1998).

A study of the singular and joint effects of maternal zinc and vitamin A deficiencies on vitamin A status and pregnancy outcomes in rats (Duncan and Hurley 1978) revealed that marginal or deficient intake of either zinc or vitamin A in dams and fetuses lowered plasma vitamin A concentrations compared with dams and fetuses with an adequate intake of each nutrient. Plasma retinol concentrations were lowest when vitamin A deficiency was accompanied by marginal-to-deficient concentrations of zinc in the diet. Another study in pregnant rats showed that supplementary dietary vitamin A did not improve the effect of zinc deficiency on vitamin A metabolism during pregnancy (Peters et al. 1986). Kanazawa et al. (2002) found that a decrease and loss of microvilli in the superficial layer of the corneal and conjunctival epithelium of rats deficient in zinc or vitamin A could be ameliorated by administration of vitamin A or zinc, respectively. In another study of rats deficient in vitamin A and zinc, impaired function of the retina during vitamin A deficiency could be reversed by repletion with vitamin A, or vitamin A and zinc, but not with zinc alone (Kraft et al. 1987).

The effects of mild vitamin A deficiency on zinc status remain unknown (Christian and West 1998). Conventional indicators of zinc or vitamin A deficiency may not be sufficiently sensitive to probe the effects of mild deficiencies in these nutrients. For example, in a study of lactating rats fed long-term with a diet marginal in zinc and vitamin A, conventional indicators did not show zinc or vitamin A deficiency (Kelleher and Lönnerdal 2002). However, although the concentrations of zinc and retinol in milk were unchanged, mammary gland metallothionein levels were increased in rats fed a vitamin A deficient diet, suggesting that marginal vitamin A deficiency may alter the zinc transport mechanism in the mammary glands of lactating rats.

Human studies

Data on the interactions between zinc and vitamin A deficiencies in humans are more limited and inconclusive than those in animals, and the effect of vitamin A deficiency on zinc metabolism is unknown. A major limitation is that plasma zinc concentration, the only routine zinc status indicator available, is not a useful indicator of zinc status because it is influenced by stress, infection, food intake, and hormonal status and only represents 0.1% of total body zinc (King and Keen 1999). Thus, of the six cross-sectional studies carried out between 1980 and 1993, only three showed a positive correlation between vitamin A metabolism and zinc status, measured as serum retinol and/or retinol binding protein and serum zinc concentrations, respectively (Christian and West 1998).

A small number of supplementation trials have also been conducted. In mildly to moderately undernourished 12–35 month old children in Bangladesh supplemented with vitamin A and/or zinc, no evidence of an interaction was observed. Combined zinc and vitamin A supplementation increased serum retinol and serum zinc concentrations, whereas supplementation with vitamin A or zinc alone improved only vitamin A or zinc status, respectively (Rahman et al. 2002b). Despite the improved vitamin A and zinc status of children receiving the combined supplementation regime, their growth velocity, height, and weight was not different from those of other groups (Rahman et al. 2002a). In the same study, however, the prevalence of persistent diarrhea and dysentery was reduced to a greater extent by combined supplementation than by single supplementation (Rahman et al. 2001).

As vitamin A and zinc play an important role in immunocompetence, Albert et al. (2003) investigated whether supplementation with vitamin A, zinc, or both would increase the vibriocidal antibody response to oral cholera vaccine in children. Although all groups showed a significant vibriocidal antibody response after vaccination, with the highest proportion in the group supplemented with both micronutrients, factorial analysis showed that zinc, but not vitamin A, had a significant positive effect with potential benefits for the cholera vaccination program.

Several other studies have looked for a synergistic effect of concurrent zinc and vitamin A supplementation. However, as with the cross-sectional studies, the results are inconsistent. For example, in studies of preschool children in Belize (Smith et al. 1999) and Bangladesh (Rahman et al. 2002b) and of school-age children in Thailand (Udomkesmalee et al. 1992), vitamin A supplementation had the same effect on

serum retinol concentrations regardless of whether it was administered in conjunction with zinc supplementation and, similarly, zinc supplementation had the same effect on serum zinc concentration regardless of whether it was given in conjunction with vitamin A supplementation. These results thus indicate that there is no synergism between zinc and vitamin A. In contrast, Rahman et al. (2002b) found that vitamin A deficiency (plasma retinol <0.7 μmol/L) was significantly less prevalent in the group supplemented with both vitamin A and zinc compared with the other groups. In pregnant women, Christian et al. (2001) found that zinc supplementation resulted in a larger increase in plasma zinc concentrations in women receiving either β-carotene or vitamin A than in those receiving placebo. In a recent supplementation trial in Indonesia, supplementation with β-carotene and zinc, but not with β-carotene alone, was effective in improving vitamin A status of both mothers and infants 6 months postpartum (Dijkhuizen et al. 2004).

In conclusion, although animal studies clearly indicate an interaction between zinc status and vitamin A metabolism and vice versa, its public health significance to human populations has not been consistently demonstrated. Further studies aimed at elucidating the interaction in human populations are unlikely to produce useful results until a more reliable zinc status indicator is developed. Alternatively, it might be possible to use morbidity or immune function end points if they could be identified. When foods are fortified or biofortified with vitamin A or provitamin A carotenoids, it would seem prudent to add zinc if there is good evidence that the diet of the target population is deficient in zinc. The addition of zinc would doubtless improve zinc status, and may help to maximize the utilization of vitamin A.

Knowledge gaps and suggestions for future research

If a population is deficient in more than one micronutrient, multiple micronutrient supplementation or multiple micronutrient food fortification is recommended (WHO/UNICEF/UNU 2001). Supplementation with multiple micronutrients may not only control the individual micronutrient deficiencies, it may also have synergistic effects arising from interactions between the micronutrients. Studies on the interaction between vitamin A and iron have produced quite compelling evidence that vitamin A deficiency decreases iron utilization, as well as clear evidence of the public health benefits of dual supplementation of iron and vitamin A to populations deficient in both nutrients so as to improve the impact of the iron on hemoglobin levels. An interaction between vitamin A and zinc also appears to occur, but its public health importance remains unclear and will likely remain that way until a better measure of zinc status is developed.

In relation to biofortification and the interactions between nutrients, it is suggested that work in this area initially focus on filling the current knowledge gaps in regard to β-carotene and iron. Knowledge of the absorption and conversion of the dietary β-carotene present in different staple foods into retinol is particularly uncertain, making it difficult to calculate the level of biofortification necessary to significantly improve vitamin A status. Most previous studies have determined the bioavailability of β-carotene by measuring increases in plasma β-carotene on consumption of fruits and vegetables, and a few by measuring the increase in plasma retinol. However, because of the variability and difficulties in interpreting such work, the way forward would appear to be to conduct additional studies using stable isotope techniques in which total body stores of vitamin A are assessed before and after intervention. It is also not known how other nutrient deficiencies influence the absorption of provitamin A carotenoids and, in particular, how nutrient deficiencies influence the conversion of provitamin A carotenoids into retinol. The necessity of dietary fat for the absorptive stage and the effect of food preparation techniques also need to be better quantified.

If β-carotene is absorbed and serum retinol increases, the evidence suggests that hemoglobin levels will rise if sufficient iron is also provided in the diet. What needs to be resolved, however, is what other nutritional and environmental factors also influence the effects of β-carotene and vitamin A on iron absorption and iron utilization for hemoglobin synthesis. Some studies have shown an increase in bioavailability, some have found no effect and, more worryingly, one study has reported that vitamin A decreases iron bioavailability in children with IDA. Given the discrepancies in the published data, clear recommendations to plant breeders are difficult to make. It is therefore recommended that further radio and stable isotope studies be carried out to investigate the influence of vitamin A, β-carotene, and other carotenoids on iron bioavailability and that stable isotope studies be conducted to determine whether vitamin A status affects the incorporation of iron into hemoglobin.

Preliminary studies indicate that iron, zinc, and β-carotene levels can be increased in staple foods by plant breeding techniques (Khush 2002). The challenge is now to show that they can be increased to nutritionally useful levels and to demonstrate that the additional micronutrients are sufficiently bioavailable as to have a positive effect on health in target populations.

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