Special Article

July 1993: 199–204

Plant Sources of Provitamin A and Human Nutriture

Noel W. Solomons, M.D., and Jesus Bulux, M.D., M.P.H.

Hypovitaminosis A is a problem in many parts of the developing world. Beyond the stop-gap measures of capsule distribution and food fortification, increased consumption of accessible sources of vitamin A, specifically of the carotenoid provitamin A in yellow, orange, and green plants, has been promoted as the sustainable, long-term solution. However, a search of the available literature reveals few examples of human studies to support the effectiveness of this solution. Evidence from feeding studies shows an almost universally poorer uptake of intact carotenoids from plant sources as opposed to pure, chemical sources. With notable exceptions, the bioconversion of plant carotenoids to preformed vitamin A also seems to be inefficient. Epidemiologic observations in poor Third World populations and in vegetarians in an industrialized nation indicate a relatively greater potency for animal sources of vitamin A. In developing countries, low fat intakes, intestinal roundworms, recurrent diarrhea, and tropical enteropathy all may contribute to reduced utilization of plant provitamin A. The accepted 6:1 equivalency of β-carotene to preformed vitamin A must be challenged and reexamined in the context of dietary plants. The consequences of operating on a miscalculation could be serious indeed for public health programs designed to alleviate and eradicate hypovitaminosis A.

Introduction

Guatemala has been classified by the World Health Organization as a nation with a high probability of a high incidence of endemic hypovitaminosis A. Given the recent implications of increased childhood mortality with mild vitamin A deficiency,[1] hypovitaminosis A is more than an academic issue for public health nutritionists working in Central America and other poor areas of the Third World. The present discussion will focus on a crucial tactical and strategic question: what dietary sources of vitamin A will provide the greatest security for both the maintenance of vitamin A nutriture and the restoration of adequate status from a deficiency state? In particular, it will reexamine assumptions and generalizations about the vitamin A equivalency of provitamin A carotenoids in natural foods.

Liver concentrations of vitamin A increase progressively with the complexity (and usually the size of the predator) of the food chain of carnivorous marine fish and mammals. This became all too evident when early Arctic explorers became acutely ill from vitamin A toxicity after consuming polar bear liver. When only animal tissue sources with preformed vitamin A are involved, prodigious amounts of vitamin A can be exchanged between predator and prey. Yet may species are strict and lifelong vegetarians, and vitamin A deficiency is not a prominent veterinary concern with herbivorous animals. The childhood joke concerning rabbits, carrots, and eyeglasses is a parable about plants as a source of dietary vitamin A. In fact, it is obvious that strict herbivores effectively extract enough retinol to avoid vitamin A deficiency. On the other hand, there is no evidence that any human has developed vitamin A toxicity from consuming rabbit liver.

The Dietary Equivalency of Carotenes in Terms of Retinol Equivalents

Early versions of the *Recommended Dietary Allowances* cited experience that in the United States about half the observed vitamin A intake was from foods of animal origin and the other half from plant foods, specifically dark green leafy vegetables and yellow and orange fruits and vegetables. When it came time to provide a common expression of dietary vitamin A as the retinol equivalent (RE), it was decided that, on a weight-for-weight basis,

Dr. Solomons is Scientific Coordinator and Dr. Bulux is Chief of the Task Force on Vitamin A and Micronutrients at the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala.

β-carotene had one-sixth the vitamin A value of a corresponding amount of pure retinol.[2] This was not based on empirical data but, rather, on two assumptions: 1) carotenes would be absorbed one-half as well as preformed vitamin A, and 2) the average extent of bioconversion is 33%.[2] A, one-half weight: weight equivalency was assigned to other carotenes (e.g., α-carotene, β-cryptoxanthin) with provitamin A activity. For the purposes of dietary calculations and dosage, the one-sixth rule reigns in nutrition, and no distinction is made between the β-carotenes in chemical form and the natural carotenoids in plants.

However, the efficiency of bioconversion of carotenoids to retinoids has little experimental verification in *humans*. This led the present authors to review the extant literature and to use our research experience to draw inferences. There is reason to question that the biologic conversion of provitamin A compounds into the active vitamin is as efficient as current dogma suggests. An error of *over*estimation would have obvious consequences in planning adequate diets and for public health intervention programs.

Observations on the Intact Uptake (Bioavailability) of β-Carotene in Human Feeding Studies

The *bioavailability* of carotenoids can be defined as their uptake in intact form from the diet into the body. A number of human studies from the US Department of Agriculture's Human Nutrition Research Laboratory at Beltsville, Maryland, recently compared the uptake of β-carotene from a purified chemical source to that occurring in natural foods.[3,4] The authors observed that the rise in plasma β-carotene that accompanied either a single dose of a 30-mg capsule of the compound or a chronic, multiple-dose daily feeding of carrots as the source of an equivalent amount of β-carotene was five- to sixfold greater with carotene in capsule form. It should be noted that these studies were conducted in well-nourished North American adults. The investigators conducted two additional confirmatory bioavailability studies, also in adults, in which carrot sources were compared to purified β-carotene.[5,6]

Coworkers from Arizona and Guatemala[7–9] have worked on developing investigative tools to quantify β-carotene bioavailability and bioconversion. In one such study,[7] either 15 or 30 mg of isolated β-carotene were provided to schoolchildren. The maximal mean postdose increases in plasma β-carotene were 8 ± 9 and 14 ± 13 μg/dL, respectively. In another group of 23 children, there was a contest to see how many cooked carrots they could eat in a single sitting. Consumption ranged from 12.4 to 950 mg of β-carotene from carrots (median: 27 mg), but the increments at the 8-hour interval were one-third those seen with the isolated chemical on a milligram-for-milligram basis.[8]

In a longitudinal study, schoolchildren from a suburb of Guatemala City received either 20 daily 6-mg doses of β-carotene in capsule form or the same amount as 50 g of cooked carrots. With the capsule form, a threefold increase was seen over initial levels of plasma β-carotene at days 10 and 20, whereas no increase was seen in subjects given the cooked carrots.[9] It should be noted that the four comparative studies discussed above relate to the uptake of *intact* carotenoids. It is a logical assumption that if carotene uptake into the intestinal cell is impeded in some way, its conversion to the active vitamin within the enterocyte might also be affected.

The *bioconversion* of carotenes involves their hydrolysis in the gut to yield the active vitamin in the form of retinal.[10] In human subjects, documenting the conversion of an oral dose of β-carotene to its derivative vitamin is a complex procedure. Maiani et al.,[11] in Italy, demonstrated a small—but detectable and quantifiable—rise in retinyl esters (the form of retinol in the postprandial chylomicrons) in the circulation of subjects receiving oral β-carotene; this suggested an index for monitoring bioconversion in a single meal in human subjects. Using this principle, Bulux et al.[12] found detectable increments (postdose rise of more than 1 μg/dL) in retinyl esters in 16 of 20 poor rural schoolchildren following a 30-mg oral dose of β-carotene in capsules, with a mean retinyl ester response of 8 ± 3 μg/dL.[12] By contrast, there was no detectable ester response after meals of carrots containing 12.4–950 mg of β-carotene in urban schoolchildren whose average retinol status was equivalent to that of subjects in the carrot study previously described.[8]

Observations on the Conversion of Oral Carotenoids to Retinoids (Bioconversion) in Human Subjects

The alternative approach to evaluating bioconversion is to feed a carotene source over a prolonged period and to monitor any changes in vitamin A nutriture. The present authors reviewed a number of carotene supplementation studies in adult subjects in which investigators have monitored plasma or serum retinol and generally found it to be stable with time.[13] This is not unexpected, however, as the subjects had balanced, omnivorous diets and adequate retinol levels to begin with.

In a few studies in Third World children, less-than-optimal retinol levels were present in some or all the subjects tested.[9,14–17] Within the limits of circulating retinol levels as an index of changes in hepatic vitamin A status, these should allow some examination of the relative efficiency of bioconversion.

The results of the Guatemalan study[12] on a 20-day feeding of provitamin A sources also could have bearing on the bioconversion paradigm. The authors compared the interval rise in circulating retinol levels with serial daily dosing of 6 mg of pure β-carotene or 6 mg of β-carotene from carrots with changes in retinal observed in children receiving either 1000 RE of vitamin A daily as retinyl palmitate or a placebo. In none of the four groups was any rise in retinol seen, suggesting that the study's short duration may have limited the ability to detect a retinol response, thereby hampering interpretation of the results.

Few groups have reported a dramatic circulating retinol response to the chronic administration of plant sources of provitamin A. One such study is that of Jalal et al.[14] in West Sumatra, Indonesia, in which a green plant-based diet was fed and the longitudinal change in retinol was monitored. As compared to a control group, significant increments in retinol were observed. Another study that purports to show evidence for bioconversion is that of Hussein and Tohmay[15] in Egypt. Of the 13 children in this study, seven received 60,000 RE of vitamin A in a single-dose retinyl palmitate capsule, four others received a cumulative dose of 25,000 RE over 40 days in the form of spinach, while two children consumed a cumulative dose of 16,000 RE over the same period in the form of carrots. Each of the groups doubled its retinol level during the interval of observation, while the total carotene levels remain unchanged. In addition to the small—and unbalanced—sample sizes and the lack of randomization, there also was no placebo control group, which could have accounted for any improvement in retinol levels deriving merely from involvement in the research.

In a later study, Hussein and Tohmay[16] placed normal adult subjects on a low-vitamin A diet for nine days and then provided various amounts of green and orange vegetables to determine the amount of these foods needed to maintain stable plasma retinol levels over a 13-day period. From this brief experiment, the authors formulated the relative and differential efficiency of bioconversion from spinach and carrots. Given the inherent stability of plasma retinol within the adequate range of concentrations and the brief periods of "depletion" and "maintenance," it is difficult to accept this as a sufficient model for assessing bioconversion efficiency in humans.

Inferences on Bioconversion Efficiency from Epidemiologic Surveys

A three-nation, multicenter study was conducted in Tanzania, the Philippines, and Guatemala to validate a rapid food-frequency instrument designed to identify community-level vitamin A deficiency.[17] Some interesting, and initially paradoxical, findings were revealed when the results from five rural hamlets in each country were compared in an ecological, epidemiologic correlation. The Guatemalan sites had the lowest intakes of dark green leafy vegetables and of orange and yellow plants, but they had the better apparent vitamin A status based on both retinol levels and xerophthalmia signs. However, in terms of serving *animal* sources of vitamin A, the Guatemalan communities had higher frequencies of intakes, and this seemed to be the differential factor. It appears that, although only semiquantitative in nature, sources of retinoids are more potent than sources of carotenoids as protective foods against hypovitaminosis A in rural populations.

Finally, cross-sectional studies in vegan and vegetarian populations in industrialized nations bear on the issue of bioconversion of plant provitamin A.[18,19] Some illustrative data on adult vegetarians come from North American vegetarians of the Seventh Day Adventist sect. Schultz and Leklem[18] calculated daily dietary intake in Seventh Day Adventists who were non-meat-eating vegetarians and compared them to Seventh Day Adventists who did eat meat and non-Adventist omnivores. The respective average vitamin A intake, calculated using the 1:6 and 1:12 factors for β-carotene and other carotenoids were, for men 1794, 1606, and 1806 RE, respectively, and for women 1646, 1322, and 1824 RE, respectively. Average intake of the Seventh Day Adventist vegetarians was similar to that of the at-large omnivores, but the authors comment that, whereas the vitamin A intake of the meat eaters was largely attributable to liver intake, for the vegetarians broccoli, carrots, squash, and cantaloupe dominated the vitamin A sources.

In French vegetarian and nonvegetarian adults, a recent study[19] attempted to correlate dietary intakes with nutritional status. The vegetarians of both genders had much higher plasma levels of α- and β-carotenoids than did omnivorous controls. Circulating levels of retinol, however, were roughly equal for both groups of women, with 3% of the vegetarians and none of the nonvegetarians having a blood retinol level below 30 μg/dL. For the men,

however, vegetarians had a mean retinol concentration of 3.4 ± 1.3 μg/dL, with 6% of the vegetarians having low levels, vs. 3.9 ± 1.2 μg/dL for the omnivores, with no at-risk levels of circulating retinol. Again in the study, female subjects had equivalent intakes across groups—1500 RE in vegetarians and 1400 RE in omnivorous women. The vegetarian men, in spite of lower plasma retinol levels, had a calculated intake of vitamin A of 1900 RE, with a contribution of 0.5 mg of preformed vitamin A and 8.0 mg from carotenoids. The omnivorous men with better vitamin A status consumed only 1400 RE daily from 0.8 mg of animal sources and 3.6 mg of plant carotenoids.

Possible Factors in Developing Countries That Reduce the Efficiency of Vitamin A Utilization from Plant Carotenoids

There are a number of theoretical—and not explicitly tested—reasons why provitamin A sources may not optimize human vitamin A reserves. First, bioconversion may not be regulated to maintain ideal hepatic reserves. For instance, if bioconversion can be up-regulated to produce a more active vitamin, this may be only a brief-burst effect, activated only in severe deficiency and turned off well before adequate reserves have been attained. Alternatively, nature may have been so concerned for the danger of vitamin A toxicity that the protective down-regulation of carotenoid bioconversion into vitamin A is initiated long before the liver can accumulate an abundant reserve of the vitamin.

Ironically, it is in populations of developing countries, those traditionally most vulnerable to hypovitaminosis A, that lower efficiency of bioconversion might be a reality. Aside from regulatory issues (above), other intrinsic (host) or extrinsic (diet) factors may act to minimize the intraintestinal transformation of provitamin A into the active vitamin. In the host domain is the health of the intestine, constantly under assault by infective agents of the tropical environment, such as recurrent diarrheal episodes, tropical enteropathy, and chronic protozoal and helminthic infections.[20] It has been shown that some of these conditions affect vitamin A absorption.[13,21,22]

In the case of parasites, Taren et al.,[22] in rural Panama, noted a strong negative association between ascaris infections and circulating retinol levels, suggesting interference by the nematode with uptake of the preformed vitamin, bioconversion of the provitamin, or both. Jalal et al.[14] found that Indonesian children with ascaris infections had much lower increases in β-carotene and retinol during prolonged feeding with a green herb-supplemented diet than did children who were free of the infection.

The low level of fats in traditional diets also can restrict maximal bioconversion of provitamin A in developing country populations. There are widespread areas of the world where fats make a minimal contribution to total energy intake. Since both the exocrine secretory mechanisms (bile salts, pancreatic lipase) and the mucosal responses (packaging of fats into lipoproteins) are stimulated and regulated by the magnitude of fat in the diet, consumption of carotene in meals with only small amounts of total fat can limit its assimilation.[23,24]

Finally, we must consider the situation of the food matrices in foods rich in carotenoids. Provitamin A sources are often high in dietary fiber and in inorganic, and poorly absorbable, iron. The theoretical influence of dietary fiber would be to microencapsulate carotene within an indigestible matrix or to bind the compound in a hydrophilic milieu that would exclude it from the mechanisms of lipid absorption. The potential influence of iron would be to act as an oxidant, destroying or isomerizing the carotenoids prior to their absorption. More refined diets with the balance of iron in the heme form, characteristic of affluent societies, may be more conducive to the bioavailability—and hence the bioconversion—of dietary carotenoids. Moreover, one could envision how carotenes presented in pure, isolated forms may be more available as precursors of vitamin A than carotenes of phyto-origin.

The Consequences of Incorrect Assumptions About the Nutritional Value of Plant Carotenoids

Proceeding now from the hypothesis that the efficiency of β-carotene bioconversion has been overestimated, at least when it comes from natural sources, it can be assumed that much more than 1.3 kcal and 115 g of Swiss chard leaves, estimated with the 1:6 assumption, would be needed to provide the true, biological equivalency of 1000 RE obtained with 105 kcal and 240 g of fortified skim milk.[25] If one assumes a further 50% reduction of efficiency as a Third World reality, this equivalency estimate for Swiss chard would rise to 2.6 kcal and 230 g. The physical (gastric) capacity to consume plants, the cultural acceptability to consumers of large serving portions of vegetables and fruits in a meal, or both, may limit the quantities of natural carotenoids that can be eaten routinely in a free-living population. The net weight and bulk of plants needed to supply a given amount of provitamin A activity may be much greater than an equivalent amount of animal-origin sources of preformed vitamin A, such as fortified milk, butter, or liver.

From the perspective of public health interventions, one must look beyond the current selection and amounts of foods to some alternative for providing more dietary vitamin A. There are three basic options: 1) more sources of the preformed vitamin, that is, foods of animal origin; 2) more sources of provitamin A, that is, foods of plant origin; or 3) food fortification of some common vehicle, such as salt, sugar, flour, monosodium glutamate, or the like, with a chemical vitamin A (or provitamin A) source. Questions about sustainability are raised with regard to the last option. The promotion of certain chronic diseases associated with the saturated fat and cholesterol content typical of animal sources of vitamin A,[26] as contrasted with the putative health-protective properties of dietary fiber and phytosterols,[27] suggests that providing vitamin A from plant sources may be the most appropriate solution. In developing countries, moreover, the differential market cost of foods of animal versus vegetable origin is an additional overriding consideration.

Despite strategic biases, however, nutritionists and public health planners must ask how much more of the plant sources of provitamin A low-income people can be coaxed into eating in a long-term, sustainable fashion. Given doubts about the true, average bioconversion efficiency, can the plant-source option promote real hepatic sufficiency of vitamin A reserves in human populations? Could it be, in fact, that instead of pushing larger amounts of yellow, orange, and dark-green, leafy plants in the diet, it will be necessary to promote retinyl palmitate fortification of a common food or to promote (and subsidize) small amounts of animal protein with high concentrations of preformed vitamin A in order to achieve physiologically efficient and logistically feasible public health interventions? The patchwork of current evidence and theoretical speculations raises doubts as to the success that can be achieved in correcting endemic hypovitaminosis A with an exclusively horticultural strategy.

Challenging the Conventional Assumptions on the Value of Dietary Plant Carotenoids as Utilizable Vitamin A

An unquestioning faith in the 1:6 bioconversion factor[2] has delayed both the expression of doubts and the empirical inquiries needed to accept or reject the null hypothesis about plant sources of provitamin A as efficient sources of the vitamin for deprived populations. This, in turn, implies directed, applied field research efforts. In the human context, the goal of providing an external supply of vitamin A is either to increase hepatic reserves or to improve nutritional status (i.e., reverse subadequate nutriture). The ethics of elective hepatic biopsies for research purposes are a barrier to the most direct assessment of human vitamin A stores, while the ethics of allowing slow and uncertain improvement of clinically apparent syndromes of hypovitaminosis A impede therapeutic dietary trials in severely deficient populations.

The inferences discussed earlier in this paper have been derived from observations using either: 1) a shift in circulating retinol as an index of total body accumulation or 2) a single-dose uptake as the gauge of intestinal handling of dietary β-carotene. The limitations of the diagnostic tools dictate the limits of firmness of our biologic conclusions.

We conclude that additional research is urgently needed to resolve the issues. Thus, despite the expense and complexity that stable isotope technology can represent, especially when used in the Third World, it would seem to hold the promise to raise the certainty of our conclusion to a higher level. Specifically, we should exploit dilution studies of stable isotope-labeled retinol as an index to monitor the total body reserves of vitamin A across the entire spectrum of nutriture as a major improvement over inferences from changes in circulating retinol. Similarly, a functional index of changes in vitamin A nutriture would be an advance over an acute uptake study in terms of the interpretation of true bioconversion. Dark adaptation procedures, modified for young children, could provide a tool for monitoring improvement in mild to moderate states of vitamin A nutriture.

1. Bellagio Brief: Vitamin A deficiency and childhood mortality. New York: Helen Keller International, 1992
2. Food and Agriculture Organization/World Health Organization. Requirements of vitamin A, thiamine, riboflavin and niacin. FAO Series no. 8. WHO Technical Report Series no. 362. Rome: FAO, and Geneva: WHO, 1967
3. Brown ED, Micozzi MS, Craft NE, et al. Plasma carotenoids in normal men after a single ingestion of vegetable or purified β-carotene. Am J Clin Nutr 1989;49:1258–65
4. Micozzi MS, Brown ED, Edwards BK, et al. Plasma carotenoid response to chronic intake of selected foods and β-carotene supplements in man. Am J Clin Nutr 1992;55:1120–5
5. Carughi A, Hooper F. Plasma carotenoid level before and after supplementation with a carotenoid complex. New York Academy of Sciences conference on carotenoids in human health, San Diego, CA, February 6–9, 1993, Abstracts 1993:13
6. Masaki K, Doi T, Yyge K, Yoshioka Y, Nagata Y. Plasma β-carotene response to intake of β-carotene drink in normal men. New York Academy of Sciences conference on carotenoids in human

health, San Diego, CA, February 6–9, 1993, Abstracts :31
7. Canfield LM, Bulux J, Quan de Serrano J, et al. Plasma response to oral β-carotene in Guatemalan schoolchildren. Am J Clin Nutr 1991;54:539–41
8. Canfield LM, Bulux J, Lima AF, et al. Plasma response to oral carotene supplementation in well-nourished Guatemalan children. FASEB J 1989;3: A465
9. Bulux J, Quan de Serrano J, Perez R, et al. Plasma levels of β-carotene after short-term feeding of Guatemalan children. FASEB J 1991;5:A1074
10. Olson JA. The provitamin A function of carotenoids. J Nutr 1989;119:105–8
11. Maiani G, Mobarhan S, Ceccanti M, et al. Beta-carotene serum response in young and elderly females. Eur J Clin Nutr 1989;43:749–61
12. Bulux J, Quan de Serrano J, Rivera C, et al. Intraintestinal bioconversion of β-carotene to retinyl palmitate in Guatemalan children is related to their retinol and β-carotene status. FASEB J 1990; 4:A3815
13. Solomons NW, Bulux J. Effects of nutritional status on carotene uptake and bioconversion. In: Canfield LM, Krinsky NI, Olson JA, eds. Carotenoids in human health. New York: New York Academy of Sciences, 1993 (in press)
14. Jalal F, Nesheim MC, Sanjur D, Habicht JP. Effects of deworming, dietary fat intake and carotenoid rich diets on vitamin A status of *Ascaris lumbricoides* infected preschool children in West Sumatra. FASEB J 1990;4:A1376
15. Hussein L, El Tohmay M. Effect of supplementation with vitamin A or plant carotenes on plasma retinol levels among young Egyptian males. Int J Vit Res 1989;59:229–33
16. Hussein L, El Tohmay M. Vitamin A potency of carrot and spinach carotenes in human metabolic studies. Int J Vit Nutr Res 1990;60:229–35
17. Sloan N, Rosen D. Validation of the HKI frequency method to identify communities with vitamin A deficiency. Abstracts XV, Meeting of the International Vitamin A Consultative Group, Arusha, Tanzania, March 1993 (in press)
18. Schultz TD, Leklem JE. Dietary status of Seventh-Day Adventists and non-vegetarians. J Am Diet Assoc 1983;83:27–33
19. Millet P, Guilland JC, Fuchs F, Klepping J. Nutrient intake and vitamin status of healthy French vegetarians and non-vegetarians. Am J Clin Nutr 1989; 50:718–27
20. Keusch GT, Solomons NW. Microorganism, malabsorption, diarrhea and dysnutrition. In: Calabrese EJ, Scherr GH, eds. Advances in human nutrition, vol 2. Park Forest, IL: Chem-Orbital, 1985: 165–209
21. Mahalnabis D, Simpson TW, Chakrabarty ML, Chameli G, Bhattacharjee AK, Mukherjee KL. Malabsorption of water miscible vitamin A in children with giardiasis and ascariasis. Am J Clin Nutr 1979;32:313–18
22. Taren DL, Nesheim MC, Crompton, et al. Contributions of ascariasis to poor nutritional status in children from Chiriqui Province, Republic of Panama. Parasitology 1987;95:603–13
23. Erdman J Jr. The physiologic chemistry of carotenes in man. Clin Nutr 1989;7:101–6
24. Dimitrov NV, Meyer C, Ullrey DE, et al. Bioavailability of β-carotene in humans. Am J Clin Nutr 1988;48:298–304
25. Bowes and Church's Food Values of Portions Commonly Used. 9th ed. Philadelphia: J.B. Lippincott, 1962
26. National Research Council. Diet and Health. Implications for Reducing Chronic Disease Risk. Washington, DC: National Academy of Sciences, 1989
27. Ziegler RG. Carotenoids, vegetables, fruits, and risk of cancer. In: Canfield LM, Krinski NI, Olson JA, eds. Carotenoids in human health. New York: New York Academy of Sciences, 1993 (in press)