

## Bioavailability of carotenoids

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**SUMMARY.** Our current knowledge about the bioavailability of provitamin A carotenoids in foods is insufficient, fragmentary and difficult to interpret. Past methods of estimating the vitamin A value of food carotenoids suffer both from uncertainty about the meaning of bioavailability and from the inadequacy of the indicators used in its determination. Reported conversion ratios of  $\beta$ -carotene to vitamin A in humans *in vivo*, depending on conditions, range from 2:1 to 26:1 ( $\mu\text{g}/\mu\text{g}$ ). Thus, the ratio of 6:1, devised by the World Health Organization, must be considered as a rough average estimate that is not applicable to all diets. Strategies to increase the dietary intake of carotenoid-containing foods should include measures to enhance carotenoid bioavailability.

**Key-words:** Carotenoids, bioavailability, vitamin A value.

**RESUMEN. Biodisponibilidad de carotenoides.** Nuestros conocimientos actuales sobre la biodisponibilidad de los carotenoides en alimentos son insuficientes, fragmentados y difíciles de interpretar. Métodos pasados para calcular el valor de vitamina A de los carotenoides alimenticios padece tanto de la incertidumbre sobre el significado del término biodisponibilidad como de la inadecuación de los indicadores usados en su determinación. Las razones reportadas para la conversión del  $\beta$ -caroteno en vitamina A en humanos *in vivo*, dependiendo de las condiciones, varían de 2:1 a 26:1 ( $\mu\text{g}/\mu\text{g}$ ). Así, la razón de 6:1, establecida por la Organización Mundial de la Salud, debe ser considerada como un estimativo promedio aproximado que no se aplica para todas las dietas. Las estrategias para aumentar el consumo de los alimentos que contienen carotenoides deben incluir medidas para realzar la biodisponibilidad de los carotenoides.

**Palabras clave:** Carotenoides, biodisponibilidad, valor de vitamina A.

### INTRODUCTION

The best utilized ingested form of vitamin A is as an ester of retinol in an oily solution. The ester passes intact through the mouth and stomach, is hydrolyzed to retinol in the upper intestine, and is absorbed as a micelle into the intestinal mucosa (1). Retinol is subsequently esterified with long-chain fatty acids, and particularly with palmitic acid, is incorporated into chylomicra, and is secreted into the lymph. Upon reaching the systemic circulation, the triglyceride of the chylomicron is hydrolyzed in large part to glycerol and fatty acids by lipoprotein lipase, and the resultant chylomicron remnant is taken up by the liver as well as, to a minor degree, by other tissues. In the liver, retinol is reesterified and stored for subsequent use, primarily in stellate cells but also in hepatocytes. Under normal dietary conditions, preformed vitamin A is absorbed very well and is stored efficiently (1).

Preformed vitamin A is found primarily in foods of animal origin (1). Vitamin A can also be formed, however, from a specific set of plant pigments known as carotenoids (1,2). Over 600 carotenoids exist in nature, of which approximately 50 serve as precursors of vitamin A. Because the vast majority of humans obtain their vitamin A from these carotenoid precursors, carotenoid bioavailability is an important public health consideration in most of the world. Carotenoids are less

bioavailable than preformed vitamin A in food. First of all, they are bound rather tightly within the matrix of the fruit or vegetable. Thus, they must be released from the matrix by digestive processes. Secondly, their requirements for absorption into intestinal cells are more demanding than for vitamin A. Thirdly, they must be enzymatically cleaved into vitamin A within intestinal cells or within other cells of the body. And, fourthly, they must be stored either as vitamin A or as carotene itself within various tissues. The bioavailability of carotenoids in foods has varied over ten-fold from one study to another (2).

At the outset, it is useful to define the concept of bioavailability (3). Absolute bioavailability is defined as the proportion of an ingested dose that is absorbed in a biologically useable form. Absolutely bioavailability can be determined fairly readily for nonmetabolizable compounds, such as metals, but is much more difficult for metabolizable organic substances like carotenoids. Thus, absolute bioavailability for carotenoids has never been determined. Relative bioavailability is the percentage of the substance that is absorbed in a biologically utilizeable form relative to a reference substance treated in the same manner. Obviously, comparative studies are much easier to conduct. The reference substance has usually been either  $\beta$ -carotene in oil or retinyl ester in a readily bioavailable form. The most commonly used indicator of bioavailability has been changes in plasma retinol in vitamin

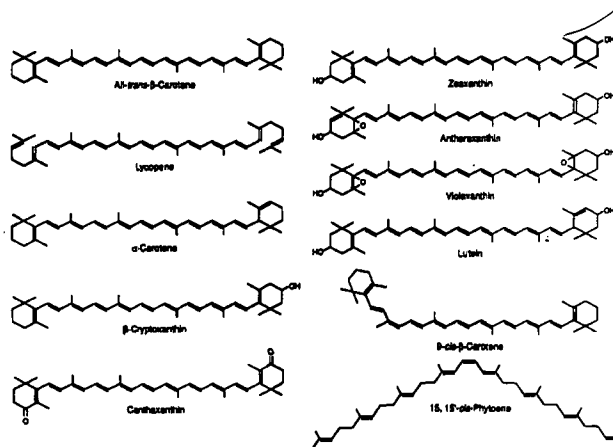
A-depleted subjects in response to increased intake of a known amount of a carotenoid.

Two facets of carotenoid bioavailability exist: Firstly, its absorption into intestinal cells and secondly, its conversion into vitamin A. In practice, these two facets are very difficult to separate. Thus, the absolute bioavailability of a single known dose of a provitamin A carotenoid is defined as the percentage amount of vitamin A-active substances formed from it. Similarly, the relative bioavailability concerns the response of an appropriate indicator to a carotenoid, usually in food, relative to a reference substance.

### Structures

The structures of some common carotenoids are shown in Figure 1 (4). The parent compound, all-*trans*  $\beta$ -carotene, is the most common provitamin A carotenoid found in foods. Its cis isomers, including 9-*cis*  $\beta$ -carotene, is less active biologically than the all-*trans* form. Furthermore, other carotenoids that contain a single  $\beta$ -ionone ring, like  $\beta$ -cryptoxanthin and  $\alpha$ -carotene, are approximately 50% as active in biological testing. The other carotenoids shown, which do not serve as precursors of vitamin A, nonetheless are common constituents of foods. Indeed, lutein and lycopene, at least in the United States, are present in plasma in higher concentrations than is  $\beta$ -carotene. These other carotenoids, which may have protective aspects relative to health, are not precursors of vitamin A (2).

FIGURE 1  
Some common naturally occurring carotenoids (4)



### Intakes

The intakes of total vitamin A, including preformed vitamin A and provitamin A carotenoids, vary tremendously in different areas of the world (5). In general, populations in most industrialized areas of the world have adequate intakes of approximately 1 mg of preformed vitamin A and 2 mg of  $\beta$ -carotene or its equivalent. In less industrialized countries, the total amount of vitamin A ingested is less, and the percentage

derived from provitamin A carotenoids is much higher. Vitamin A deficiency, as indicated by clinical manifestations, has most commonly been found in Africa and Asia, although, at one time, certain areas of Central America and South America were also affected. Although clinical vitamin A deficiency tends to be decreasing in most countries of the world, the World Health Organization nonetheless estimates that the health of more than 250,000,000 preschool children is compromised by vitamin A inadequacy (5,6).

### Cleavage

The bioavailability of carotenoids initially depends on the way in which they are converted into vitamin A or other products. Carotenoids can be cleaved centrally to yield, in the case of  $\beta$ -carotene, two molecules of retinal.  $\beta$ -Carotene might also be cleaved eccentrically to yield  $\beta$ -apo-carotenals, which then can be oxidatively converted to one molecule of retinal. Finally, carotenoids can be oxidatively destroyed by free radicals and by enzymes such as lipoxygenase. In healthy individuals with an adequate vitamin A status, the major pathway for the formation of vitamin A from provitamin A carotenoids is central cleavage, which is carried out by an enzyme,  $\beta$ -carotene-15,15'-dioxygenase, that is present in the cytosol of the intestine and of many other organs (7,8). The properties of this enzyme are given in Table 1. Because of its instability, the enzyme has not yet been highly purified nor cloned.

TABLE 1  
Properties of  $\beta$ -carotene 15,15'-dioxygenase  
(EC 1.13.11.21)

Cleaves many provitamin A carotenoids
Cytosolic
Requires molecular oxygen
Gives two moles of retinal per mole of $\beta$ -carotene cleaved
$K_M = 1-10 \mu M$
$pH_0 = 7.5-8.5$
Requires a detergent
Needs free sulfhydryl groups
Probably contains iron or copper
Present in both neonatal and adult intestinal cells, liver and many other tissues
Associated with CRBP-II concentration

### Equivalencies

As a result of growth responses in vitamin A deficient rats, an international unit (IU) was defined as 0.3  $\mu g$  of all-*trans* retinol in oil or 0.6  $\mu g$  of all-*trans*  $\beta$ -carotene in oil (9). Thus, in *in vivo* studies, 1  $\mu g$  of all-*trans* retinol was equivalent to 2  $\mu g$  of all-*trans*  $\beta$ -carotene. A two-fold difference therefore exists between the cleavage ratio, where 1  $\mu g$  of  $\beta$ -carotene yields an equivalent amount of all-*trans* retinol, and the ratio *in vivo*, where twice as much  $\beta$ -carotene is required. This difference can be attributed to the poorer intestinal absorption

of  $\beta$ -carotene relative to vitamin A and the conversion of a discrete portion of the retinal formed from  $\beta$ -carotene to retinoic acid, which is not usually measured and is rapidly metabolized. These findings in animals were confirmed in humans in a vitamin A depletion-repletion study with male volunteers (10).

As already indicated,  $\beta$ -carotene in foods is less biologically active than  $\beta$ -carotene in oil. Thus, in 1967 the World Health Organization (WHO) suggested that a ratio of 6  $\mu\text{g}$  all-*trans*  $\beta$ -carotene in food is equivalent to 1  $\mu\text{g}$  all-*trans* retinol in food (11). The retinol equivalent (RE) was thereby established, which could be expressed in any mass units, i.e., ng,  $\mu\text{g}$ , mg, etc. Generally, the  $\mu\text{g}$  RE is most employed. The WHO committee based their equivalency ratio on two assumptions; namely, (a) that the mass ratio of the maximum conversion of  $\beta$ -carotene in oil, its most bioavailable form, to vitamin A *in vivo* was 2:1 and (b) that the bioavailability of  $\beta$ -carotene from foods on the average was one-third that of  $\beta$ -carotene in oil. Thus,  $2 \times 3 = 6 \mu\text{g}$   $\beta$ -carotene per  $\mu\text{g}$  retinol. Other common carotenoids, like  $\beta$ -cryptoxanthin and  $\alpha$ -carotene, were considered to be half as active as  $\beta$ -carotene. *Cis*-isomers were later considered as well to be half as active as their all-*trans* counterparts.

The WHO expert committee based their 3:1 ratio primarily on balance studies in which the fecal content of carotenoids was subtracted from the amount ingested to give the amount absorbed. This method tends to overestimate the amount of the carotenoid absorbed because of the poorer extraction efficiency of carotenoids from foods than from oil and of probable destruction of carotenoids in the GI tract. Nonetheless, the expert committee did make a correction for these confounding factors. In support of their conclusions, mass conversion ratios of 5.8:1 and 6.4:1 for  $\beta$ -carotene in papaya and in amaranth, respectively, can be calculated from the results of a well-designed study with preschool children in India (12).

On the basis of many similar studies conducted in India, a conversion ratio of 4:1 for the food based  $\beta$ -carotene/vitamin A conversion was accepted as part of the Indian dietary allowances (13). The approach used was similar to that employed by the WHO, but the average bioavailability of  $\beta$ -carotene in ingested vegetables and fruits, relative to  $\beta$ -carotene in oil, was considered to be 50%, not 33.3%.

In 1995, lactating Indonesian women were fed either stir-fried vegetables containing 3.5 mg of  $\beta$ -carotene and 7.8 g of fat, an enriched wafer containing the same amount of  $\beta$ -carotene in a highly bioavailable form together with fat, or a control wafer containing no  $\beta$ -carotene (14). In those receiving the enriched wafer, serum retinol, breast milk retinol and serum  $\beta$ -carotene increased significantly, whereas in those given either stir-fried vegetables or the control wafer, no positive response was noted. Thus, the public health strategy of enhancing the intake of carotenoid containing fruits and vegetables as a means of improving vitamin A status was questioned. Later, in a similar study carried out with Indonesian

children, ratios of 26  $\mu\text{g}$  of  $\beta$ -carotene in vegetables or 12  $\mu\text{g}$  of  $\beta$ -carotene in fruits were calculated to be equivalent to 1  $\mu\text{g}$  of retinol on the basis of changes in serum retinol (15). These latter ratios are probably too high, inasmuch as no correction was made for absorbed but not immediately converted provitamin A carotenoids. Indeed, in some cases, up to 60% of absorbed  $\beta$ -carotene is not immediately converted into vitamin A (16).

Another way of looking at this issue is to compare the intake of carotenoids to the basal vitamin A requirement of growing children. The basal vitamin A requirement, as defined by FAO/WHO, is the amount of vitamin A that is needed for growth and the maintenance of various physiological responses but without leading to significant vitamin A storage (17). In the case of 3- to 5-year old children, the basal requirement is approximately 216  $\mu\text{g}$  retinol equivalents. In feeding studies involving amaranth and papaya in India, in which ratios of 5.8 and 6.4  $\mu\text{g}$   $\beta$ -carotene/ $\mu\text{g}$  retinol were obtained, the basal vitamin A requirement is fully met (12). On the other hand, if ratios of 26:1 for vegetables or 12:1 for fruits are used (15), only 1/4 or 1/2 of the basal vitamin A requirement would be met by the provided diets. These children, however, grew normally and plasma retinol values rose under this feeding regimen (12).

A variety of new methods are now being used to gain greater insight into carotenoid bioavailability. These include area-under-the-curve studies with isotopically labelled  $\beta$ -carotene, vegetables and vitamin A (18); sophisticated kinetic models involving a variety of compartments after the ingestion of labeled  $\beta$ -carotene (19); careful area-under-the-curve measurements of carotenoids and vitamin A in the triglyceride-rich fraction of plasma after a single dose of  $\beta$ -carotene in oil or foods (16); the measurement of total body stores of vitamin A employing isotope dilution techniques before and after increased ingestion of carotenoid-rich foods (20); and steady-state isotope-dilution procedures with labeled carotenoids and vitamin A (21).

### Influences

Several factors influence the conversion of  $\beta$ -carotene and other provitamin A carotenoids into vitamin A. These factors might be considered in two groups; namely: (a) those that influence the activity of the major cleavage enzyme,  $\beta$ -carotene-15,15'-dioxygenase, and (b) those that influence in large part the absorption of provitamin A carotenoids from the GI tract.

$\beta$ -Carotene-dioxygenase shows increased activity in the presence of high intakes of fat and particularly of polyunsaturated fat, high intakes of protein and high intakes of  $\beta$ -carotene (22,23). The activity is increased as well when the vitamin A status is poor. The enzyme activity is decreased by factors opposite to those just cited.

Exogenous factors that influence carotenoid bioavailability (2,24) are summarized in Table 2. The absorption efficiency of all-*trans*  $\beta$ -carotene in oil is usually 20-60%. The absorption

efficiency declines rapidly, however, as the amount ingested increases. In general, hydrocarbon carotenoids are fairly well absorbed as are xanthophylls and apo-carotenoids. The all-*trans* isomers clearly are better absorbed than the *cis* isomers and diepoxides and allene carotenoids tend to be poorly absorbed. When present together in the GI tract, carotenoids tend to compete with each other for absorption (25,26). Thus,  $\beta$ -carotene seems to inhibit canthaxanthin and lutein absorption, at least when present together in large amounts, but may enhance lycopene absorption. Oxo-carotenoids seem to affect  $\beta$ -carotene absorption to a lesser degree.

TABLE 2  
Factors affecting carotenoid bioavailability

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* Amount ingested
* Carotenoid structure
* Competition among carotenoids
* Physical state
* Dietary fat, fiber, oxidants and antioxidants
* Food preparation and particle size
* Stomach acidity and the composition and flow of bile
* Lipid malabsorption
* Infections
* Genetic factors

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The physical state of the carotenoid is a key issue, in that carotenoids in oil or in specially prepared beadlets are very well absorbed, whereas those in fruits and vegetables are absorbed to a lesser degree. The presence of dietary fat and antioxidants in the GI tract enhances  $\beta$ -carotene stability and absorption, whereas fiber and oxidants have the opposite effects. Cooked, pureed carrots and spinach seem to be absorbed approximately 3-fold better than the raw, intact vegetables (27). In some instances, this ratio can be even higher. A reduced rate of bile flow, a decreased amount of cholic acid analogs and stomach acidity will also influence carotenoid absorption.

Any of a series of lipid malabsorption syndromes will of course markedly reduce the absorption of carotenoids (1,2). Infections, either systemic or from intestinal parasites, will also adversely affect carotenoid uptake. Finally, genetic factors clearly influence all aspects of carotenoid and vitamin A metabolism. These factors, however, have not been quantitated in any effective way.

#### FINAL REMARKS

The bioavailability of food carotenoids as sources of vitamin A is affected by a host of endogenous and exogenous factors. Clearly, no single conversion factor suitable for all foods and conditions can be defined. Ways of preparing and storing carotenoid containing foods to enhance their bioavailability should be devised and taught to populations at

risk. Quite possibly, different conversion ratios might be employed for dark green leafy vegetables, fruits, carotenoids in oily solutions such as red palm oil, and so on, in order to better estimate the actual bioavailability of carotenoids in a diet. Clearly, the carotenoid content of ingested foods in a given culture might well differ markedly from values found in food composition tables (28). Nonetheless, with the application of new experimental approaches, better information concerning this important nutritional issue should be forthcoming in the near future.

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#### REFERENCES

- Ross AC. Vitamin A and retinoids. In: Shils ME, Olson JA, Shike M, Ross, AC, editors. Modern nutrition in health and disease, 9th ed. Baltimore: Williams & Wilkins, 1999: 305-27.
- Olson JA. Carotenoids. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease, 9th ed. Baltimore: Williams & Wilkins, 1999: 525-41.
- Parker RS. Bioavailability of carotenoids. Eur J Clin Nutr 1997; 51: 86-90.
- Olson JA, Krinsky NI. The colorful, fascinating world of the carotenoids: important physiologic modulators. FASEBJ 1995; 9: 1547-50.
- Underwood BA. Vitamin A in human health: public health considerations. In: Sporn MB, Roberts AB, Goodman DS, editors. The retinoids: biology, chemistry, and medicine. New York: Raven Press, 1994: 211-27.
- Underwood BA, Arthur P. The contribution of vitamin A in public health. FASEB J 1996; 10: 1040-8.
- Devery J, Milborrow BV.  $\beta$ -carotene-15,15'-dioxygenase (EC 1.13.11.21): isolation, reaction mechanism and an improved assay procedure. Brit J Nutr 1994; 72: 397-414.
- Nagao A, During A, Hoshino C, et al. Stoichiometric conversion of all-*trans*  $\beta$ -carotene to retinal by pig intestinal extract. Arch Biochem Biophys 1996; 328: 57-63.
- Moore T. Vitamin A. Amsterdam: Elsevier, 1957.
- Sauberlich HE, Hodges RE, Wallace DI, et al. Vitamin A metabolism and requirements in the human studied with use of labeled retinol. Vitam Horm (USA) 1974; 32: 251-75.
- Food and Agriculture Organization/World Health Organization. Requirements of vitamin A, thiamine, riboflavin, and niacin. Report of a joint FAO/WHO Expert Group. FAO Nutr Meetings Rep Ser No. 41. Rome: FAO Press, 1967: 15-27.
- Devadas P, Saroja S, Murthy NK. Availability of  $\beta$ -carotene from papaya fruit and amaranth in preschool children. Indian J Nutr Dietet 1980; 17: 41-4.
- Gopalan C, Narasinga Rao BS. Dietary allowances for Indians. ICMR Sp Rep Ser No 60. New Delhi: ICMR Press 1991: 46-53.

14. De Pee S, West CE, Muhilal, Karyadi D, Hautvast JGAJ. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. *Lancet* 1995; 346: 75-81.
15. de Pee S, West CE, Permaesih D, et al. Orange fruit is more effective than are dark-green leafy vegetables in increasing serum concentrations of retinol and  $\beta$ -carotene in school children in Indonesia. *Am J Clin Nutr* 1998; 68: 1058-67.
16. van Vliet T, Schreurs WHP, van den Berg H. Intestinal  $\beta$ -carotene absorption and cleavage in men: response of  $\beta$ -carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of  $\beta$ -carotene. *Am J Clin Nutr* 1995; 62:110-6.
17. Food and Agriculture Organization/World Health Organization. Requirements of vitamin A, iron, folate, and vitamin B12. Report of a joint FAO/WHO expert consultation. FAO Food & Nutr Ser No 23. Rome: FAO Press, 1988: 16-32.
18. Tang G, Grusak MA, Qin J, et al. Vitamin A activity of spinach determined by isotope reference method. Abstracts, 19th IVACG meeting, 1999: 44.
19. Novotny JA, Dueker SR, Zech L, et al. Compartmental analysis of the dynamics of  $\beta$ -carotene metabolism in an adult volunteer. *J Lipid Res* 1995; 36: 1825-38.
20. Ribaya-Mercado JD, Mazariegos M, Tang G, et al. Assessment of total body stores of vitamin A in Guatemalan elderly by the deuterated-retinol-dilution method. *Am J Clin Nutr* 1999; 69: 278-84.
21. van Lieshout M, West CE, Lugtenburg J, et al. Quantification of bioavailability of  $\beta$ -carotene and its bioconversion to retinol using [ $^{13}\text{C}_{10}$ ]- $\beta$ -carotene and [ $^{13}\text{C}_{10}$ ]-retinyl palmitate. Abstracts, 19th IVACG meeting 1999: 42.
22. van Vliet T, van Vlissingen MF, van Schaik F, van den Berg H.  $\beta$ -Carotene absorption and cleavage in rats is affected by the vitamin A concentration of the diet. *J Nutr* 1996; 126: 499-508.
23. During A, Nagao A, Terao J.  $\beta$ -Carotene-15,15'-dioxygenase activity and cellular retinol-binding protein type II level are enhanced by dietary unsaturated triacylglycerols in rat intestine. *J Nutr* 1998; 128: 1614-9.
24. de Pee S, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. *Eur J Clin Nutr* 1996; 50: S38-S53.
25. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and  $\beta$ -carotene when administered to human adults in separate or combined oral doses. *Am J Clin Nutr* 1995; 62: 604-10.
26. Paetau I, Chen H, Goh NM-Y, White WS. Interactions in the postprandial appearance of  $\beta$ -carotene and canthaxanthin in plasma triacylglycerol-rich lipoproteins in humans. *Am J Clin Nutr* 1997; 66: 1133-43.
27. Rock CL, Lovalvo JL, Emenhiser C, et al. Bioavailability of  $\beta$ -carotene is lower in raw than in processed carrots and spinach in women. *J Nutr* 1998; 128: 913-6.
28. Rodriguez-Amaya DB, Amaya-Farfan J. Current status of analytical methods for measuring provitamin A. *Arch Latinoam Nutr* 1992; 42: 180-91.