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Bioavailability Of β-Carotene From Plant Foods

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From time immemorial, plant foods containing β-carotene have been a major dietary source of vitamin A in Third World countries. Ever since the β-carotene of plant foods came to be known to act as a precursor of vitamin A, several studies have been carried out in experimental animals and human volunteers to measure the bioavailability of β-carotene from carotene-rich foods.

Bioavailability of nutrients from foods implies the efficiency with which the nutrients present in the food are utilised for performing their biological functions in the body. The utilisation of nutrients from food involves mainly two processes: (1) release of nutrients into the gut lumen from the food matrix following digestion, and (2) the uptake of nutrients by the mucosal cell and their transport into the body.

A series of events including competitive interaction between molecules can promote or inhibit the intestinal release of nutrients and their uptake by the mucosal cells. As far as the carotenoids present in plant foods are concerned, several factors can facilitate or inhibit their biological utilisation; the ease with which carotenoids present in the cell-matrix of the plant food are released in the gut will be influenced by the processing of the food (cooking, etc) by which means the cell may be ruptured. Since carotenoids are lipid-soluble they have to be emulsified to form mixed micellae in which pancreatic lipase and bile salts from the liver, under appropriate conditions of ionic strength, pH and molarity, allow it to reach the mucosal cell surface. Fat in the diet plays an important part, both in the release of nutrients from the plant cell-matrix and their emulsification, to be followed by their being taken up by mucosal cells and transported via the lymph as chylomicrons.

Several other events can also prevent the intestinal uptake of carotenoids, including their destruction through oxidation which is influenced by oxidants notably, non-haeme iron, pH, ionic strength and oxygen tension in the stomach as well as the intestine. Presence of antioxidants in food can protect against such destruction.

Carotenoids also can be lost due to binding to insoluble substances such as dietary fibre besides incomplete release from the food matrix. The provitamin A carotenoid content can be reduced by its bioconversion to active retinol in the mucosal cell. There could also be competitive interactions between and among carotenoids with regard to their uptake by the intestinal mucosal cells. Such competition also exists between provitamin A carotenoid species and their isomers. Researches into the above aspects of bio-availability of carotenoids may provide valuable leads for augmenting the bioavailability of carotenoids from plant foods. Available information on the bioavailability of β-carotene from plant foods is briefly discussed here.

EXPERIMENTAL STUDIES

In animal studies on bioavailability, young growing animals (mostly rats) made vitamin A deficient were fed diets containing carotene-rich test foods, and the growth and vitamin A stores in liver determined as a measure of carotene utilisation. The results were compared with those obtained with a standard dose of retinol or pure β-carotene fed to a control set of animals[1].

Studies in humans have adopted two approaches: (1) the chemical balance study on habitual diets containing the plant source of carotene or pure β-carotene; and (2) the assessment of the effect of feeding carotene-rich foods to vitamin A deficient children on their plasma levels of retinol.

Balance studies: There have been a number of balance studies since the 1930s to measure carotene absorption from different carotene-containing foods in different countries including India. The joint FAO/WHO Expert Group[2] which went into the question of human requirements for vitamins, including vitamin A in 1967, reviewed the results of all the

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TABLE 1
Absorption of Carotenes from Different Food Sources in Human Subjects

Source	Subjects		Per cent absorption		Ref
			β-carotene	Total carotene	
Purified β-carotene	Adult males	(4)	98.8	–	8
Amaranth leaves	"	(4)	75.7	58.1	"
Papaya fruit	"	(4)	90.9	45.8	"
Diet with different sources of carotene	"	(4)	81.1	35.5	"
Carrots	Boys	(7)	"	46.6	14
Spinach	"	(3)	"	80.7	"
Pure β-carotene	Preschool children	(15)	85.4	–	6,7
Amaranth leaves	"	(4)	70.2	–	6
"	"	(15)	61.4	–	7
Fenugreek leaves	"	(5)	59.1	–	4
Drumstick leaves	"	(5)	61.0	–	4
Leaf protein concentrate	"	(15)	76.7	–	7
Papaya fruit	"	(8)	77.0	–	5
Spirulina	School children	(6)	74.6	–	9

Figures within parenthesis indicate the number of children studied.

published studies on carotene balance from different foods. The reported carotene absorption values were found to spread over a wide range (vide Appendix 3, p74 of the report). The Expert Group, on the basis of the then available carotene absorption data, arrived at an average figure of 33 per cent for carotene absorption from plant foods to establish the conversion factor for arriving at the Retinol Equivalent (RE) of dietary carotene and its intake to meet vitamin A requirements. The suggested conversion factor of 1 μg β-carotene = 0.167 μg retinol is widely accepted and used for estimating dietary carotene intake to meet daily vitamin A requirements.

STUDIES IN INDIA

Carotene absorption studies were also carried out in India subsequently, using locally consumed green leafy vegetables (GLVs)[3]. The results of some of these and other studies[4-10] summarised in Table 1, will show that carotene absorption from GLVs is in fact much higher than the levels assumed by the FAO/WHO Expert Group. On an average, 50 per cent of carotenes with provitamin A activity in GLVs are absorbed by adult men as well as by children. The Expert Group of the ICMR[11] which recommended dietary allowances for Indians, therefore, adopted a value of 50 per cent for the absorption of carotene from Indian diets containing carotene-rich plant foods. The recommended conversion factor was 1 μg β-carotene = 0.25 μg retinol.

Some doubts have been raised about carotene absorption figures, as some of the unabsorbed carotene excreted in the faeces might have undergone bacterial degradation, thus leading to an underestimation of excretion and an overestimation of absorption. It must be pointed out, however, that in all these studies absorption of pure β-carotene incorporated into the diet had also been measured concurrently, and absorption of over 90 per cent (85-98 per cent) had been observed for pure β-carotene. Relative to pure β-carotene absorption, the currently employed figures for dietary carotene bioavailability are thus valid and acceptable.

Studies on children: The other approach to assess the availability of dietary β-carotene as a source of vitamin A was to feed β-carotene-rich foods to children with vitamin A deficiency for a couple of weeks and follow their serum retinol levels. The increase in serum retinol levels in the experimental subjects was taken as evidence of absorption and utilisation of dietary carotene as a retinol source. It can be seen from the results of these studies[5,6,9,12,13] presented in Table 2, that feeding 1,200 μg of carotene in the form of GLVs was able to bring about an improvement in the vitamin A status of children suffering from vitamin A deficiency.

A note of caution, however, is necessary while employing this approach in assessing the bioavailability of carotene from carotene-rich foods as a source of vitamin A. If such foods are fed to vitamin A deficient subjects with low liver stores and low plasma retinol levels, elevation of plasma levels of retinol can easily be demonstrated (Table 2), as has been shown in a number of studies. However, in subjects with good liver stores and normal serum retinol levels, feeding carotene or even retinol at RDA levels may not result in an elevation of plasma retinol, even if the carotene is absorbed and converted into retinol and stored in the liver. Results of some studies[10,14,15] which illustrate this point are shown in Table 3. Absence of an increase in serum retinol level, therefore, cannot be interpreted as evidence of the unavailability of dietary carotene. Plasma retinol is an insensitive index of vitamin A status because it is only responsive to extremes of deficiencies and toxicities[14].

Epidemiological evidence: Besides the foregoing experimental evidence for the bioavailability of dietary carotene, epidemiological data too lend further support. In communities which are consuming adequate quantities of carotene-rich foods as a major source of dietary vitamin A, vitamin A deficiency is rarely seen. For example, in certain regions of India such as Jammu and Kashmir, Punjab and Haryana[16] and among tribal communities in the North East and Madhya Pradesh[17], vitamin A deficiency is quite rare because of the high level of consumption of a wide range of GLVs. On the other hand, in the eastern and southern parts of India where carotene-rich foods are only infrequently consumed, vitamin A deficiency among children is quite commonly encountered[18].

Wide seasonal differences in the prevalence of vitamin A deficiency

TABLE 2
Effect of Feeding GLVs and Other Sources of Carotenes on Serum Retinol Levels in Indian Children

Subjects	β-carotene source	Carotene fed, µg/dl	Duration of feeding (days)	Serum levels of retinol (µg)			Ref
				Initial	Final	Change	
1. Undernourished preschool children (6)	Control	Nil	15	24.7	22.5	-2.2	6
(a) with serum levels <25 µg/dl	Amaranth leaves	1,200	11	15.4	28.0	12.6	"
(b) >25 µg/dl	"	"	"	31.2	37.5	6.2*	"
(c) pooled	"	"	"	21.9	31.9	10.0	"
2. Slum children (5)	Amaranth	1,200	60	13.9	28.7	14.8	5
" (8)	Papaya fruit	"	"	13.2	29.1	15.9	"
" (10)	Retinol	300	–	12.5	35.2	22.7	"
" (5)	Control	–	"	12.8	13.2	0.4	"
3. Undernourished children (29)	Amaranth	1,040	90	21.6	30.6	9.0	12
4. Orphanage children	Spirulina			21.4			9
5. Pooled (32)	"	1,200	30	21.4	30.3	8.9	"
serum level <20 (12)	"	"	"	14.8	29.3	14.5	"
serum level >20 (20)	"	"	"	25.4	30.9	5.5	"

Figures within parenthesis indicate the number of children studied.
** Not significant.*

signs corresponding to differences in the availability of GLVs provide additional evidence.

The fact that even in those areas where vitamin A deficiencies are seen in a small minority of the population, the overwhelming majority don't show evidence of vitamin A deficiency, in spite of the fact that their diets hardly contain any food sources of preformed vitamin A would indicate that in real-life situations, among poor communities, adequate levels of vitamin A are being derived from such carotene-rich foods as are available.

It may also be mentioned that in the developed countries of Europe and North America, generous consumption of β-carotene-rich foods like GLVs and other vegetables is now being vigorously promoted, since the absorbed β-carotene from these foods is known to act as an antioxidant to protect against diseases such as epithelial cancers, which are believed to be caused by free radical damage.

Thus, on the basis of well established data on the satisfactory bioavailability of carotene from carotene-rich foods, particularly GLVs, several programmes are in operation in Third World countries to combat vitamin A deficiency by promoting the consumption of locally available GLVs and other carotene-rich foods, as also in developed countries to combat epithelial cancers.

FALLACY OF SOME RECENT CLAIMS

It is in this context that we must examine some recent claims which tend to throw doubt on the efficacy of GLVs in the prevention and control of vitamin A deficiency.

There have been two recent publications which claim that GLVs because of poor bioavailability of carotene are ineffective in improving vitamin A status, and that therefore, alternative strategies for ensuring vitamin A nutrition are necessary. The claims run counter to the massive evidence to the contrary already available in literature; and could prove misleading and adversely influence current programmes for combating vitamin A deficiency through promotion of GLVs. Objective scientific scrutiny should reveal that these claims are untenable and not valid.

In the study reported by Sesika de Dee, *et al*[19], there was a marked difference in the effective level of intake of β-carotene (based on the actual content of RE) in the two groups under comparison. In view of the well-known differences with respect to the bioavailability as between pure β-carotene and β-carotene from foods, the demonstration of lack of identical response between the two groups given identical doses proves nothing new; nor does it establish that GLVs, for the reason that the β-carotene contained in them is not 100 per cent bioavailable, are no good in the prevention of vitamin A deficiency.

It is also possible, indeed it is reasonable to expect, that in the vegetable supplemented group in the above studies in which each subject was getting nearly as much as 150 g vegetable daily as supplement, the subjects may not have been consuming their habitual levels of vegetables in their daily home diets during the study period unlike in the wafer supplemented groups. The authors, however, claim that there was no change in the normal dietary habits of their subjects on the basis of a single diet survey carried out during the supplementation period employing the 24-hour recall method, which the authors themselves admit is not very reliable. The authors' claim in this regard appears unlikely to be correct. If the vegetable supplement had resulted in a decrease in the normal intake of vegetables in the home diet in the vegetable group, unlike in the wafer group, the lack of change in the serum retinol levels is to be expected.

TABLE 3
Comparison of Serum Retinol Responses to Retinyl Acetate, Pure β-carotene and Carotene-rich Vegetables in Boys with Different Initial Levels of Serum Retinol

Study subjects	Vitamin A source		No of days fed	Daily supplement of β-carotene/ retinol (μg)	Mean serum retinol level (μg/dl)			Ref
					Initial	Final*	Change	
Boys	Retinol	(7)	40	1,500	17.2	32.5	15.3	10
(Egypt)	Spinach	(4)	"	1,750	17.2	33.4	16.2	"
	Carrots	(2)	"	1,181	20.0	40.0	20.0	"
School-age children (Guatemala)	Retinol	(17)	20	1,000	35.2	38.1	2.9	15
	Carrot	(17)	"	6,000	36.8	37.9	1.9	"
	Pure β-carotene	(16)	"	6,000	34.3	31.7	-2.6	"
	Placebo	(17)	"	–	34.9	39.6	4.7	"
Vitamin A deficient	Carrots	(1)	"	6,000	18.6	33.2	14.6	"
School-age children	"	(1)	"	"	23.2	43.2	20.0	"
(Guatemala)								

Figures in parenthesis indicate the number of subjects studied.
** Values obtained from figures in references 10 and 15.*

The reported lack of significant increase in serum retinol levels is also to be expected. The majority of the subjects studied had normal vitamin A status and had serum retinol levels around 25 μg/dl. Only 3 per cent of the subjects had serum levels of 10 μg/dl, considered to indicate deficiency, while 33 per cent had levels between 10-12 μg/dl, indicating marginal deficiency and a majority of the subjects, that is, 64 per cent had normal serum levels of retinol.

As pointed out earlier, subjects with normal serum levels of retinol do not respond to small doses of retinol or β-carotene. Hence, it is not surprising that the subjects in the vegetable supplemented group with an effective intake of only 350 μg RE of β-carotene per day from leafy vegetables did not respond with any significant increase in serum retinol levels. This will not rule out the fact that they have in fact, benefited.

The study of Bulux, *et al*[15], among Guatemalan children also suffers from the same flaw. Children with serum levels of 35 μg/dl in the Guatemalan study did not respond with any increase in serum levels when fed for 20 days with 6 mg of pure β-carotene or carrots providing 6 mg β-carotene or 1,000 RE of retinyl palmitate (Table 3). Indeed this was to be expected.

In contrast to the above two studies, the study at NIN[6] where the effects of feeding amaranth to preschool children as a source of β-carotene on serum retinol was tested, it was observed that while serum retinol levels increased significantly in those children with serum retinol levels <25 μg/dl, the increase in those with serum levels >25 μg/dl, was small and insignificant (Table 2). Plasma retinol level is reported to be an insensitive index of vitamin A status because it is only responsive at extremes of deficiency or toxicity[14]. Because carotenes are apparently first metabolised in the intestine and then esterified to retinyl esters for export to the liver, postprandial serum retinol may provide a sensitive measure of the effectiveness of β-carotene as a source of retinol only in vitamin A deficient children[4-12]. In line with this, the two children in the study of Bulux, *et al*[15], with very low levels of initial plasma retinol concentration (18.6 and 23.2μg/dl) who received a carrot supplement (50 g containing 6 mg carotene) for 20 days showed an almost twofold increase in plasma retinol (33.2 and 43.2 μg/dl respectively) after 20 days (Table 3). If the response of the serum retinol levels in the three groups reported in the present study of Sesika de Dee, *et al*[19], are analysed according to their respective serum retinol levels, different conclusions would emerge. Subjects who had serum levels <20 μg/dl would have shown a good response even in the vegetable group.

The conclusion drawn by the study that GLVs are an ineffective source of vitamin A, stands convincingly refuted by the glaring fact that in these studies the study population, reported to be habitually consuming the same type of vegetables as were tested in the studies, had satisfactory vitamin A status! This would confirm that they were obtaining adequate vitamin A from the leafy vegetables they were habitually consuming in their usual diets. If, on the other hand, the conclusion of the ineffectiveness of GLVs as a source of vitamin A drawn by the authors was true, the study population should have exhibited widespread vitamin A deficiency with lower levels of serum retinol than those reported.

CONCLUSION

Undeniably there are limits to the bioavailability of carotene from vegetable foods; but the point to remember is that the relative lower bioavailabilty of carotene in vegetables need not necessarily imply that vegetables are *ineffective* in combating

vitamin A deficiency. Given their levels of bioavailabilty, GLVs in amounts in which they can be easily consumed in daily diets can still provide the daily vitamin A requirement. In addition, they will also provide other nutrients such as folic acid, vitamin C, iron, zinc and other bioactive phytochemicals. A tablet of a single nutrient — β-carotene or vitamin A or indeed a cocktail of synthetic nutrients — cannot mimic the total effect of food. The findings of the Finnish study (ATBC study[20-22]) had demonstrated the inefficiency of a mixture of alpha-tocopherol and β-carotene supplements to afford protection against lung cancer, in contrast to the positive protective effect reported with vegetables and fruits (especially of yellow and green varieties[23,24]). Also, while the unregulated intake of single nutrients could occasionally cause imbalance and deleterious side effects, natural foods will be devoid of such undesirable side reactions.

A sensible public health policy for the prevention of vitamin A deficiency in poor communities must, therefore, squarely rest on the optimal use of foods rich in β-carotene. Synthetic nutrients may be reserved for *clinical* use in extreme cases of severe deficiency and no more.

Our efforts must be directed not towards discarding GLVs and towards promoting the use of synthetic vitamin A, but towards further improving the bioavailability of β-carotene from carotene-rich food sources through: (1) identifying and selectively propagating varieties rich in β-carotene; (2) improving culinary procedures in order to avoid cooking losses and to ensure maximal retention of nutritive value; and (3) promoting acceptability and increased intake of GLVs through nutrition education.

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NUTRITION NEWS

The 29th Annual Meeting of the Nutrition Society of India will be held from November 21-22, 1996, at the National Institute of Nutrition, Hyderabad. The highlights of the meeting are:

- Symposia on: 'Nutrition Education' and 'Recent Trends in Infant Feeding'.
- Twentieth Gopalan Oration by Dr John D. Potter on: 'Plant Foods and Cancer Risk — Science and Tradition'.
- Eighth Srikantia Memorial Lecture by Dr N. Kochupillai on: 'Micronutrient Deficiency and Human Health and Development'.

October 16: **World Food Day Symposium** at the FAO Regional Office, Bangkok. Dr C. Gopalan will speak on: 'Fighting Hunger and Malnutrition in South and South East Asia — the Emerging Agenda for the Turn of the Century'.

December 6-9: International Symposium organised by **The Rank Prize Funds**, UK, on: 'Feeding a World Population of More than Eight Billion People: A Challenge to Science'. Dr Gopalan will participate.

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