Carotenoids
Volume 5: Nutrition and Health

Chapter 8

Carotenoids as Provitamin A

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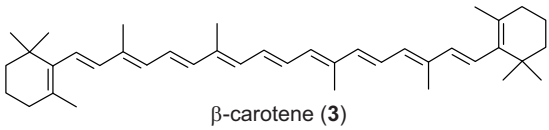
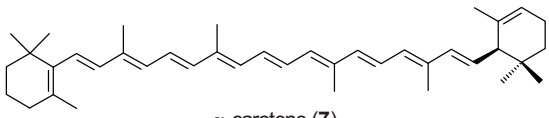
A. Introduction

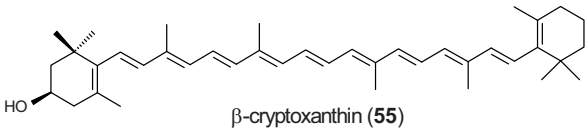
In 1930, Moore discovered that β-carotene (**3**) could be converted *in vivo* into vitamin A [1]. Since then, the vitamin A values of β-carotene and other provitamin A carotenoids, particularly α-carotene (**7**) and β-cryptoxanthin (**55**), have been investigated by various techniques.



β-carotene (**3**)



α-carotene (**7**)



β-cryptoxanthin (**55**)

As discussed in *Chapter 9*, vitamin A nutrition is of worldwide interest; deficiency of the vitamin remains a problem in developing countries, affecting 75 to 140 million children [2]. Deficiency of vitamin A (VAD) can result in visual malfunction such as night blindness and xerophthalmia [3], and can impair immune function [4], resulting in an increased incidence and/or severity of respiratory infections, gastrointestinal infections [5], and measles [6]. Vitamin A levels in HIV-positive children are lower than those in HIV-negative children [7].

Humans obtain vitamin A from their diets. In developing countries, provitamin A carotenoids in vegetables and fruits may provide more than 70% of daily vitamin A intake [8]. In contrast, in Western societies, where sources of the pre-formed vitamin A, *i.e.* eggs, meat, fish, and dairy products, are consumed extensively, provitamin A carotenoids derived from plants may provide less than 30% of daily vitamin A intake [9]. In extensive programmes to reduce or prevent clinical vitamin A deficiency in developing countries, doses of chemically synthesized vitamin A have been given periodically to populations at risk, and this has been demonstrated to be an efficient and safe strategy [10-14]. However, supplementation programmes rely on periodic mass distribution, which is difficult to sustain because of high distribution costs. Food-based interventions to increase the availability of foods rich in provitamin A have been suggested as a realistic and sustainable alternative to tackle vitamin A deficiency globally [15], but the efficacy of carotenoid-rich foods in the prevention of vitamin A deficiency has been questioned in some recent studies [16,17].

In Western populations, interest in studying the vitamin A value of dietary carotenoids has been aroused after epidemiological data have shown that diets rich in carotenoid-containing foods are associated with reduced risk of certain types of chronic diseases such as cancer [18], cardiovascular disease [19], age-related macular degeneration [20,21] and cataract [22,23] (see *Chapters 13-15*). Any disease-preventing activity of β-carotene and other provitamin A carotenoids could be ascribed either to their conversion into retinoids or to activity as intact molecules. The results of several human intervention studies, however, indicate that high-dose supplementation with β-carotene, either alone [24] or with vitamin E [25] or with vitamin A [26], does not decrease the risk of cancer or cardiovascular disease, and might even be harmful to smokers or former asbestos workers. Thus, it may be that β-carotene and other carotenoids may be health-promoting when taken at physiological levels in foods, but may have adverse properties when given in high doses and under highly oxidative conditions. The issue of the efficiency of conversion of provitamin A carotenoids into vitamin A and other retinoids is therefore of interest in both developing and developed countries.

As is well known, after an oral dose of β-carotene, both intact β-carotene and its metabolite, retinol (*1*), can be found in the circulation. In humans, conversion of β-carotene into vitamin A takes place in the intestine and in other tissues. The ratio of the amount of β-carotene given in an oral dose to the amount of vitamin A derived from this β-carotene dose is defined as the β-carotene to vitamin A conversion factor.

B. Conversion into Vitamin A *in vitro*

As illustrated in Fig. 1 and described in detail in *Volume 4, Chapter 16*, two pathways have been proposed for the conversion of β-carotene into vitamin A in mammals. The central cleavage pathway [27,28] leads to the formation of two molecules of vitamin A aldehyde (retinal, *2*) and hence vitamin A itself, retinol (*1*) from one β-carotene molecule by cleavage of the C(15,15') double bond, whereas the excentric pathway leads to the formation of a single molecule of retinal (and thus retinol) by a stepwise oxidation of β-carotene beginning at another of the double bonds of the polyene chain [29,30].

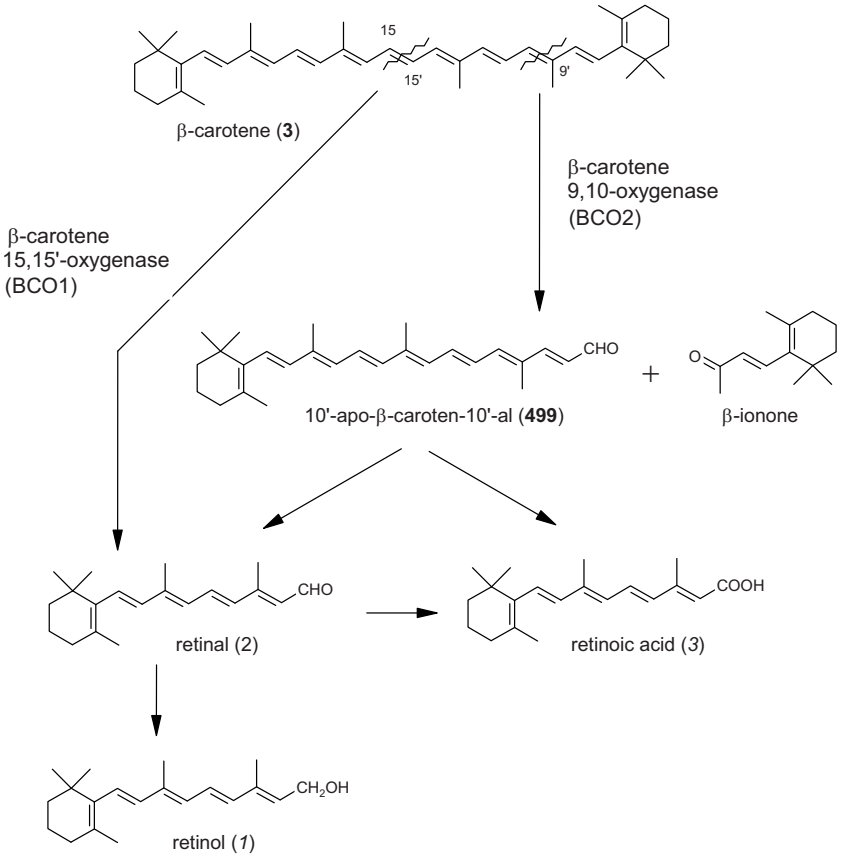
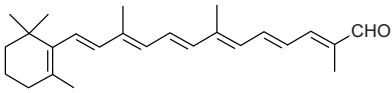
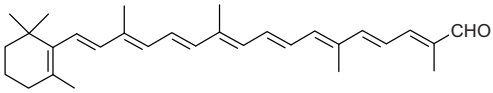


Fig. 1. The formation of vitamin A (retinol, *1*), retinal (*2*) and retinoic acid (*3*) by central or excentric cleavage of β-carotene (**3**) by β-carotene 15,15'-oxygenase (BCO1) and β-carotene 9,10-oxygenase (BCO2), respectively.

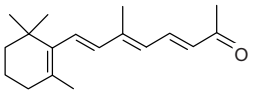
A β-carotene 9,10-oxygenase has been identified. The enzymic conversion of β-carotene into retinoic acid (*3*), retinal (*2*), 12′-apo-β-caroten-12′-al (**507**), 10′-apo-β-caroten-10′-al (**499**), and 8′-apo-β-caroten-8′-al (**482**) by mammalian tissues *in vitro* has been demonstrated [31]. In addition, the appearance of the metabolites 13-apo-β-caroten-13-one (C_{18}-ketone, *4*) and 14′-apo-β-caroten-14′-al (**513**), formed in significant amounts during the incubation of mammalian tissues with β-carotene, has been reported [32]. A recent study confirmed that both central and excentric cleavage of β-carotene take place in the post-mitochondrial fraction of rat intestinal cells, but the relative activity of the two pathways depends on the presence or absence of an antioxidant such as α-tocopherol [33].



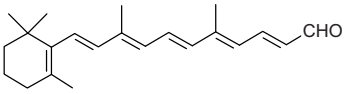
12'-apo-β-caroten-12'-al (**507**)



8'-apo-β-caroten-8'-al (**482**)



'C_{18}-ketone' (*4*)



14'-apo-β-caroten-14'-al (**513**)

In 2000, the enzyme β-carotene 15,15′-oxgenase that cleaves β-carotene to retinal was identified in chicken intestinal mucosa and subsequently sequenced and expressed in two different cell lines [34]. In addition, the existence of different types of cleavage enzymes of β-carotene in mouse [35] and human [36] was reported. Very recently, both central (β-carotene 15,15′-oxygenase, BCO1) and excentric (β-carotene 9,10-oxygenase, BCO2) cleavage enzymes have been reported in small intestine, liver, skin, eye, and other tissues [36]. The existence of at least two different β-carotene oxygenases makes estimation of the vitamin A value of β-carotene complex. The genes and enzymes, their regulation and the reaction mechanisms are discussed in *Volume 4, Chapter 16*.

C. The Conversion of Provitamin A Carotenoids into Vitamin A *in vivo*: Methods to Determine Conversion Factors

In relation to the value of β-carotene and other carotenoids as dietary precursors of vitamin A, a key and controversial question concerns the efficiency of the enzymic conversion of the carotenoids into vitamin A *in vivo*. The many food tables that list the precise carotenoid content of fruit and vegetables (see *Chapter 3*) tell only part of the story; the efficiency with which the body can obtain vitamin A from these sources is another vital factor. The absorption, transport and other factors that influence the bioavailability of carotenoids are described in *Chapter 7*. Many different numerical values (conversion factors) have been reported for the formation of vitamin A from β-carotene and other provitamin A carotenoids, either obtained from the diet or provided as supplements, and several different methods have been used to determine these conversion factors. The most useful of these methods, and the results obtained by their use, are described and evaluated below.

1. Measuring radioactivity recovered in lymph and blood after feeding radio-isotopically labelled β-carotene

A few studies have been carried out to investigate the conversion rate of radioactive β-carotene in humans. Two early studies [37,38] reported the absorption and conversion of β-carotene in adult subjects. An oral dose of labelled β-carotene was given and thoracic duct lymph was collected. In one study [37], the total radioactivity recovered in the lymph of two adult subjects given a labelled β-carotene dose was 8.7% and 16.8%. Of this, 22-30% of the absorbed radioactivity was recovered in β-carotene, and 61-71% in retinyl esters. In another study [38], the mean total radioactivity recovered in the lymph of four adult patients after taking a labelled β-carotene dose was 23.1% (range 8.7-52.3%). In this case, 1.7-27.9% of the absorbed radioactivity was recovered in β-carotene, and 68.2-87.9% in retinyl esters (one outlier was omitted). From these results, it is reasonable to speculate that the absorption of pure β-carotene in humans is in the range of 10-20%, and that about 70% of the absorbed radioactivity from labelled β-carotene is recovered in retinyl esters.

In recent years, the development of very sensitive accelerator mass spectrometry (AMS) has made it possible to use minute doses of [^{14}C]-β-carotene to study the presence of metabolites of [^{14}C]-β-carotene in human plasma, urine and faeces samples [39]. The absorption of the β-carotene was estimated at 43%, and 62% of this absorbed β-carotene was converted into vitamin A. Vitamin A values of 0.53, 0.62, and 0.54 mol from 1 mol of β-carotene were calculated, though a number of assumptions were made in the calculation, *e.g.* that 77% of absorbed β-carotene is cleaved through excentric cleavage [39,40].

2. Measuring the repletion doses of β-carotene and vitamin A needed to reverse vitamin A deficiency in vitamin A depleted adults

A depletion study [41] was conducted on sixteen healthy subjects between the ages of 19 and 34 years (seven additional subjects served as positive controls). After twelve months of depletion, only three of the subjects were vitamin A deficient; both a blood concentration below 0.35 μmol/L (10 μg/dL) and deterioration in dark adaptation were used to define 'unmistakably deficient' subjects. Of the three subjects with these 'unmistakable' signs of vitamin A deficiency, two were given β-carotene and one was given pre-formed vitamin A. Daily doses of 1,500 μg of β-carotene or 390 μg of retinol for 3 weeks to 6 months were sufficient to reverse vitamin A deficiency in these subjects. Therefore, from this human study, the β-carotene:vitamin A equivalence was determined to be 3.8:1 by weight. In 1974, another extensive and well controlled vitamin A depletion-repletion study in human subjects was reported [42]. Eight healthy male subjects between 31 and 43 years of age were depleted in vitamin A within 359-771 days. Depletion was defined by a plasma retinol level below 0.3 μmol/L (10 μg/dL) and clinical signs of vitamin A deficiency (dark adaptation impairment, abnormal electroretinogram, or follicular hyperkeratosis). Five subjects were then given vitamin A and three subjects given β-carotene. Daily doses of 600 μg retinol or 1200 μg of β-carotene were required to cure vitamin A deficiency. In this study, the β-carotene to vitamin A equivalence was, therefore, 2:1 by weight. In these studies, all subjects had been made deficient in vitamin A, so it cannot be determined whether a 3.8 μg or 2 μg equivalence of β-carotene to 1 μg of retinol is applicable in vitamin-A-sufficient individuals.

The results of earlier studies in 1939 and 1940 [43,44] are in question because of the lack of standardization of the experimental approaches and endpoints.

On the basis of these investigations with synthetic β-carotene in humans, and the lack of any precise data on the bioavailability or bioconversion of carotenoids from foods, the availability of β-carotene from the diet has been taken as one-third of the provitamin A carotenoids ingested, with a maximum conversion of absorbed β-carotene of 50% on a weight basis [9]. Since other provitamin A carotenoids (α-carotene, β-cryptoxanthin, *etc*.) can provide one molecule of vitamin A, they are expected to exhibit approximately half the vitamin A activity of β-carotene [45]. Therefore, the retinol equivalence of carotenoids in food has generally been assumed and accepted as being: 6 μg of (all-*E*)-β-carotene, or 12 μg of other provitamin A carotenoids are equivalent to 1 μg of retinol (1 retinol equivalent, RE) [9,46,47]. By using these assumptions, the NHANES (National Health and Nutrition Examination Survey) of 1970-1980 in the U.S. showed that the median adult dietary intake of vitamin A was 624 RE, with *ca*. 25% coming from carotenoids and *ca*. 75% coming from preformed vitamin A sources, as calculated from food composition tables [9] and the conversion factor of 6:1 for β-carotene to retinol conversion.

3. Measuring changes of serum vitamin A levels after feeding synthetic β-carotene or food rich in provitamin A carotenoids

There are several reasons why the vitamin A activities of provitamin A carotenoids provided in food had not been studied quantitatively in humans until recently. It was found that plasma β-carotene concentration could not be altered by eating a meal containing up to 6 mg of β-carotene in a food matrix [48,49]. Therefore, doses of unlabelled β-carotene of 6 mg or less could not be used to study β-carotene absorption or conversion, because of the insensitivity of the blood response. Past studies reported that supplementation with 12-180 mg of β-carotene is required to investigate the blood or chylomicron β-carotene response in humans [48-50]. The conversion of β-carotene into vitamin A cannot be estimated accurately in well-nourished humans by assessing changes in serum retinol after supplementation with unlabelled β-carotene, because newly-formed retinol cannot be distinguished from retinol derived from body reserves; it is well known that blood retinol concentrations are homeostatically controlled in a well-nourished individual. Nevertheless, many investigations with populations who normally have low vitamin A intake have reported blood retinol responses to acute or chronic β-carotene supplements [16,17,51]. Changes in serum retinol levels were seen [52] in vitamin A deficient (~ 0.7 μmol/L) anaemic schoolchildren aged 7-11 years, who were fed one of four supplements: (i) 556 RE/day from retinol-rich foods, n = 48; (ii) 509 RE/day from fruits, n = 49; (iii) 684 RE/day from vegetables, n = 45; or (iv) 44 RE/day from low-retinol and low-carotene foods, n = 46. The supplements were fed six days per week for 9 weeks, and the changes in serum retinol were then assessed to determine a relative conversion efficiency of β-carotene from vegetables or fruits compared with that from food rich in preformed vitamin A (egg, chicken liver, fortified margarine, and fortified chocolate milk). Those consuming fruit (diet ii) or vegetables (diet iii) showed increases of 0.12 μmol/L and 0.07 μmol/L, respectively, in serum retinol whereas the group consuming foods rich in preformed vitamin A (diet i) showed an increase of 0.23 μmol/L. The relative mean conversion factor of vegetable β-carotene into retinol was calculated, by weight, as 26:1 and that of β-carotene from orange-coloured fruit as 12:1. Use of a similar approach [53] showed that, for breast-feeding women, the conversion factors of β-carotene into retinol were, by weight, 12:1 for fruit and 28:1 for green leafy vegetables.

4. Measuring changes in body stores of vitamin A after feeding dietary provitamin A carotenoids (paired DRD test)

As shown in the previous Section, for populations with marginal to normal vitamin A status, the changes of serum retinol may not be a sensitive indicator of vitamin A status. Instead, isotope dilution techniques can be used to measure changes of total body stores of vitamin A. A deuterated retinol dilution (DRD) method was used in a study of children with marginal to normal vitamin A status, who participated in a food-based intervention with either green-

yellow vegetables or light-coloured vegetables with low carotene content [54]. The serum carotenoid concentrations of children fed green-yellow vegetables increased, whilst the serum concentration of vitamin A did not change. In contrast, the isotope dilution tests carried out before and after the vegetable intervention showed that the body stores of vitamin A were stable in the group fed green-yellow vegetables, but decreased in the group fed light-coloured vegetables. Over a 10-week period, a loss of 7 mg vitamin A from body stores was seen in the children fed light-coloured vegetables containing little β-carotene, but 275 mg β-carotene from green-yellow vegetables prevented this loss. From this paired DRD test, it was calculated that 27 μg β-carotene from vegetables was equivalent to 1 μg retinol. This conversion factor is similar to that reported in other studies for carotenoids from vegetables [54].

The paired DRD technique has also been used [55] to measure change in the vitamin A pool size after 60-day supplementation with 750 RE/day as either retinyl palmitate, β-carotene, sweet potato, or Indian spinach, compared with a control containing no retinol or carotene. Vitamin A equivalency factors of 6:1 for β-carotene in oil, 10:1 for β-carotene in Indian spinach, and 13:1 for β-carotene in sweet potato were determined.

A recent study used mixed-vegetable intervention and the paired DRD test to measure the changes in vitamin A pool size [56]. The results showed that the conversion factors were better than 12:1 for β-carotene and 24:1 for other provitamin A carotenoids.

5. Measuring intestinal absorption by analysis of postprandial chylomicron fractions after feeding synthetic β-carotene or food rich in provitamin A carotenoids

In another approach, postprandial chylomicron (PPC) response curves of β-carotene and retinyl esters in blood were measured following a single dose of β-carotene supplement in oil or from vegetables [57-59]. In these studies, triacylglycerol-rich lipoproteins (TRL) with density less than 1.006 g/mL were separated and analysed to evaluate the absorption efficiency of β-carotene (intact and, after central cleavage, as retinyl palmitate). The TRL fraction of blood lipoprotein contains both VLDL (very low density lipoproteins) and chylomicrons. However, the postprandial TRL fraction contains mainly chylomicron particles. The efficiency of absorption of β-carotene by each subject was calculated by measuring the areas under the curve (AUC, nmol.h/L) of β-carotene and retinyl ester concentrations in postprandial TRL fractions collected hourly. These curves were compared with hypothetical AUC after an intravenous dose of the same amount of β-carotene, assuming that the β-carotene disappearance follows a first-order elimination from blood with a chylomicron remnant half-life of 11.5 min [58]. Total absorption of β-carotene was measured as the sum of the AUC of β-carotene and retinyl palmitate, with the assumption that 1 molecule of β-carotene is converted into 1 molecule of retinyl palmitate.

On the basis of a postprandial chylomicron (PPC) study in ten young men aged 20-24 years [57], the mean absorption of 15 mg β-carotene (as 10% water-soluble beadlets) was reported as 17% (2.6 mg), and the conversion of absorbed β-carotene into retinyl palmitate as 52-83% (1.6 mg). A similar approach [59] in six men and six women aged 20-25 years gave a value of 8% (3.2 mg) for the mean absorption of β-carotene from a capsule of palm oil extract containing 40 mg β-carotene, while the conversion of absorbed β-carotene into retinyl palmitate was 40% (1.3 mg). These studies showed relatively similar β-carotene AUC responses, but up to a two-fold discrepancy in the reported AUC values for retinyl esters formed from the β-carotene dose, possibly due to variable recovery of the TRL fraction and the dynamic nature of chylomicron secretion and clearance. When a similar approach was used to evaluate the utilization of β-carotene from vegetables [59], little or no β-carotene response was observed in the TRL fraction after equivalent doses of 15 mg carotenoids from cooked carrots, tomato paste, or spinach were given. Thus, the suitability of the PPC method for studying the conversion of a normal dietary level of β-carotene from food is uncertain.

To compensate for the variability of TRL recovery, deuterium-labelled vitamin A has been used [60] as an extrinsic standard. A subject was given raw carrots containing 9.8 μmol (5 mg) β-carotene and 5.2 μmol (2.8 mg) α-carotene together with 7 μmol (2 mg) [$^{2}H_{4}$]-retinyl acetate, and the concentrations of β-carotene, α-carotene, and labelled and unlabelled retinyl esters in the TRL were measured at various time points up to 7 hours. With the assumption that absorption of labelled retinyl acetate was about 80% of the dose, it was calculated that 0.8 μmol of the carrot β-carotene was absorbed intact and that 1.5 μmol of unlabelled retinyl esters were formed from the carrot dose. The mass equivalency of carrot β-carotene to vitamin A was, therefore, 13:1 (without considering the contribution from 5.2 μmol of α-carotene to vitamin A). If the contribution of α-carotene is considered, the ratio is higher (16:1), assuming that α-carotene has half the activity of β-carotene.

6. Measuring blood response kinetics after feeding β-carotene labelled with stable isotopes

a) Single dose

For studying the absorption and conversion of β-carotene in humans, a sensitive method involving administration of β-carotene labelled with either [^{13}C]-β-carotene or [^{2}H]-β-carotene and analysis by MS has been used [60-64]. In one study, 1 mg of per-labelled [$^{13}C_{40}$]-β-carotene was given to a middle-aged male subject. The isotope ratios were determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) [61]. On a molar basis, 64% of the absorbed [^{13}C]-β-carotene in the circulation was recovered in retinyl esters, 21% in retinol, and 14% in intact β-carotene.

In another study [62], 73 μmol (*ca*. 40 mg) of [$^2H_8$]-β-carotene was given to a male subject, and plasma samples were drawn over a 24-day period. The isotope ratio of [$^2H_8$]-β-carotene:unlabelled β-carotene in plasma was determined. A strong correlation was reported between the ratios of [$^2H_8$]-β-carotene:unlabelled β-carotene in the plasma determined by either lengthy HPLC or MS/MS methods. The HPLC method, however, was able to detect as little as 1.87 pmol of [$^2H_8$]-β-carotene, whereas the detection limit for the MS/MS method was 100 pmol. Compartmental analysis [63] of these data showed that 22% of the β-carotene dose was absorbed, 17.8% as intact β-carotene and 4.2% as retinol. That is, 1 μg dietary β-carotene was equivalent to 0.054 μg retinol. When 37 μmol [$^2H_6$]-β-carotene (*ca*. 20 mg) and 30 μmol [$^2H_6$]-retinyl acetate (10 mg) were fed to eleven healthy, non-smoking, female subjects aged 19 to 39 years, only six of the volunteers showed a measurable response (≥ 0.01 μmol.h/L for [$^2H_3$]-retinol and/or [$^2H_6$]-β-carotene) to the labelled β-carotene dose [64]. The mean absorption of intact [$^2H_6$]-β-carotene was 6.1% for the six normal responders and <0.01% for the five non-responders. The mean absorption of [$^2H_6$]-β-carotene as [$^2H_3$]-retinol was not reported, but the data indicate that *ca*. 10% of the total absorbed [$^2H_6$]-β-carotene was converted into [$^2H_3$]-retinol. The lower absorption value found in this study was attributed to the use of doses that were neither 'solubilized nor emulsified' [64].

b) Multiple doses

To analyse [^{13}C]-labelled β-carotene, an LC/ESI-MS (liquid chromatography/electrospray ionization-mass spectrometry) method was developed [65] with a detection limit for β-carotene between 1 and 2 pmol. However, the response of ESI-MS *versus* β-carotene concentration was not linear. Later, an LC/APCI-MS (liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry) method was developed [66] and used to study the metabolism of [$^{13}C_{10}$]-β-carotene in children 8-11 years of age who had been given multiple doses of [$^{13}C_{10}$]-β-carotene, mostly as *Z* isomers, (80 μg/day) and retinyl palmitate (80 μg/day) to reach an enrichment plateau in the circulation (plateau isotope enrichment technique). The results showed that 2.4 μg β-carotene (mostly as (*Z*)-β-carotene) in oil could be converted into 1 μg retinol [66]. In another study that used HPLC/APCI-MS to determine the enrichment of intact β-carotene from a deuterium-labelled β-carotene dose, the detection limit of β-carotene was 50 pg [67]. Thus, physiological doses of β-carotene could be used to study the absorption of labelled β-carotene in humans.

c) Use of labelled retinyl acetate as a reference

An isotope reference method to determine the retinol equivalence of β-carotene in humans was also developed [68,69]. By using a known amount of [$^2H_8$]-retinol as a reference and comparing its blood response to the amount of [$^2H_4$]-retinol formed *in vivo* from [$^2H_8$]-β-

carotene, the vitamin A value of the vitamin A precursor or a food can be determined. This 'isotope reference method' can be used to define in humans the vitamin A activity of various vitamin A precursors, *e.g.* synthetic β-carotene or provitamin A carotenoids in vegetables, fruits or algae, as shown in Fig. 2.

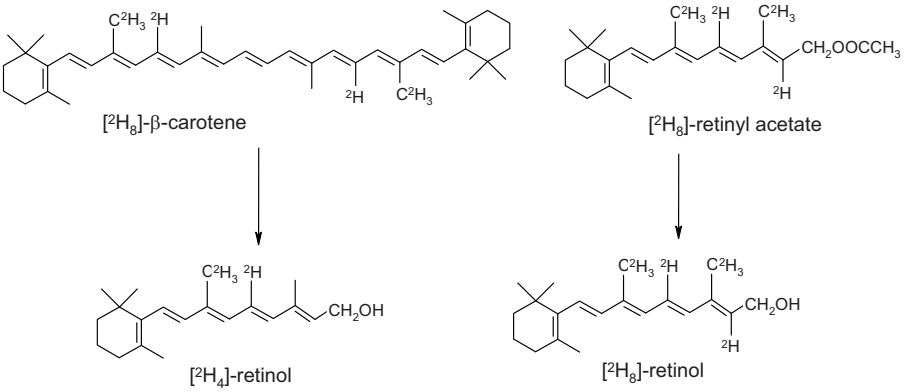


Fig. 2. Scheme to illustrate the origin of [$^2H_4$]-retinol and [$^2H_8$]-retinol detected in serum after feeding [$^2H_8$]-β-carotene and [$^2H_8$]-retinyl acetate.

In one study, two dosage levels (a pharmacological dose, 126.0 mg [$^2H_8$]-β-carotene, and a physiological dose, 6.0 mg [$^2H_8$]-β-carotene) were used 2.5 years apart in an adult female volunteer to study dose effects on the conversion of β-carotene into vitamin A [68]. Blood samples were collected over 21 days. β-Carotene and retinol were extracted from serum and isolated by HPLC. The retinol fraction was converted into a trimethylsilyl ether derivative [69], which was analysed by GC/ECNCI-MS (gas chromatography/electron capture negative chemical ionization-mass spectrometry). The [$^2H_4$]-retinol response in the circulation reached a peak 24 hours after the [$^2H_8$]-β-carotene dose was given, with a higher percent enrichment after the physiological dose than after the pharmacological dose. From this, it was calculated that 6 mg of [$^2H_8$]-β-carotene (11.2 μmol) was equivalent to 1.6 mg of retinol (*i.e.* 3.8 mg of β-carotene was equivalent to 1 mg of retinol), whereas 126 mg of [$^2H_8$]-β-carotene (235 μmol) was equivalent to 2.3 mg of retinol (*i.e.* 55 mg β-carotene was equivalent to 1 mg retinol). These results demonstrate the feasibility of using a stable isotope reference method to study the retinol equivalence of provitamin A carotenes, and show that there is an inverse dose-dependent efficiency of bioconversion of β-carotene into retinol.

The bioavailability of 6 mg (11.2 μmol) synthetic [$^2H_8$]-β-carotene was studied in 22 adult subjects (10 men and 12 women). To avoid possible absorption competition between [$^2H_8$]-β-carotene and [$^2H_8$]-retinyl acetate, the two tracers were given separately [70]. On day 1, the

subjects were given 6 mg of [$^2H_8$]-β-carotene in corn oil with a high-fat liquid beverage (25% total energy was from fat). Serum samples were collected at 0, 3, 5, 7, 9, 11, and 13 hrs after the [$^2H_8$]-β-carotene dose. On days 2 and 3, fasting serum samples were collected. Then, on day 4, volunteers were given 3.0 mg (8.9 μmol) of [$^2H_8$]-retinyl acetate (equivalent to 2.6 mg retinol) in corn oil with the same high-fat liquid beverage as was used on day 1. Serum samples were collected from 0 to 13 hrs (as on day 1) after the dose of [$^2H_8$]-retinyl acetate was given. From days 5 to 10, daily fasting serum samples were collected. After day 10, subjects were free living and their fasting serum samples were collected weekly for 8 weeks. Serum samples were analysed by HPLC and GC/ECNCI-MS. A representative serum response of the [$^2H_4$]-retinol from the [$^2H_8$]-β-carotene doses and the [$^2H_8$]-retinol from the reference [$^2H_8$]-retinyl acetate is presented in Fig. 3. The AUCs of [$^2H_4$]-retinol and [$^2H_8$]-retinol percent enrichment response for all subjects were obtained and a conversion factor for each subject was calculated. The values ranged between 2.4:1 and 20.2:1, with an average of 9.1:1 (by weight). In a similar study conducted in a healthy Chinese population, the same conversion factor of 9.1:1 was observed, with a range from 3.8:1 to 22.8:1 [71].

In a similar study of a subject given 30 μmol of [$^2H_6$]-β-carotene (16.2 mg) and a reference dose of [$^2H_6$]-retinyl acetate (10.2 mg) in olive oil (11 g), 15.9 μg of β-carotene was found to be equivalent to 1 μg of retinol [72).

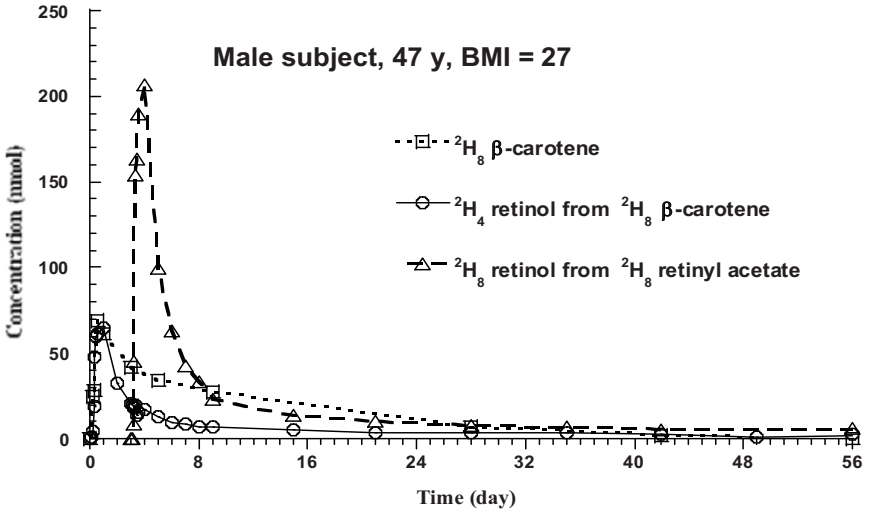


Fig. 3. Graph to illustrate the changes in concentration of [$^2H_8$]-β-carotene, [$^2H_4$]-retinol and [$^2H_8$]-retinol in serum of a male subject, age 47 years, after feeding [$^2H_8$]-β-carotene on day 0 and [$^2H_8$]-retinyl acetate on day 4. BMI: body mass index.

7. Feeding intrinsically labelled dietary provitamin A carotenoids in food

It has been common practice to assess the vitamin A value of a food from the amounts of preformed vitamin A and provitamin A carotenoids contained in that food. As discussed in Section **D** and in *Chapter 7*, major factors that affect the bioavailability of food carotenoids and the bioconversion of food carotenoids into vitamin A in humans are the food matrix, food preparation, and the fat content of a meal. Absorption of carotenoids and vitamin A from various food matrices has not been well studied because, until recently, isotopically labelled foods that can be fed to humans were not available. Therefore, in order to achieve an accurate assessment of carotenoid bioabsorption and a subsequent vitamin A value from a food source, food material is required in which the carotenoids have been endogenously or intrinsically labelled with a low abundance stable isotope. This allows presentation of the carotenoids in their normal cellular compartments, and the isotopic label makes it possible to identify those serum carotenoids (or derived retinol), which come from the specific food in question.

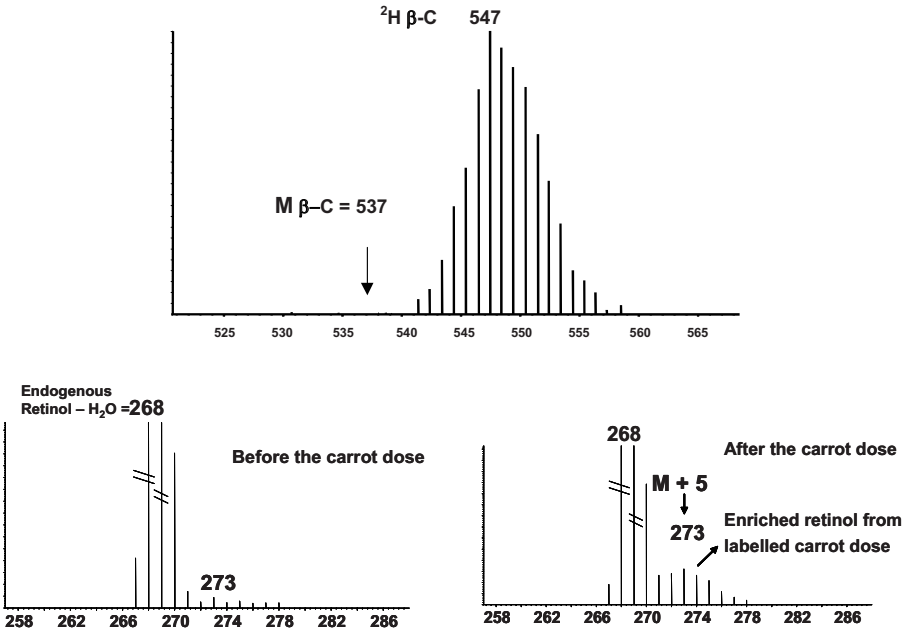


Fig. 4. Top: molecular ion region of the mass spectrum of β-carotene isolated from carrots grown in water enriched with ^2H_2O (25 atom %). A range of pseudo-molecular ions $[M+1]^+$ is seen, the most abundant being that at *m/z* 547, due to $[^2H_{10}]$-β-carotene. Bottom left: detail of the mass spectrum of unlabelled retinol, showing the $[M-H_2O]$ ion at *m/z* 268. Bottom right: detail of the mass spectrum of retinol formed after consumption of $[^2H]$-enriched carrots containing $[^2H]$-labelled β-carotene, of which the main species is the $[^2H_{10}]$ isotopomer.

Plant carotenoids can be intrinsically labelled either with ^{13}C from $^{13}CO_2$, or with ^{2}H from $^{2}H_2O$. To achieve high enrichments of the carotenoid pool, the plants must be maintained on a constant supply of the isotope throughout their entire growth period. Labelling with $^{13}CO_2$ requires a closed atmospheric system that can be regulated for humidity, temperature, CO_2 and O_2 concentrations. For $^{2}H_2O$ labelling, on the other hand, plants can easily be grown hydroponically [73] on a nutrient solution with a fixed ^{2}H atom percentage. No special facilities for the growth system are required but, by enclosing hydroponically labelled plants in a closed chamber in which the atmospheric water vapour is also enriched with $^{2}H_2O$, improved labelling is achieved, and costs can be reduced by recovering transpired water *via* a condensing system. Water with 25% atom excess of ^{2}H generates a range of isotopomers of carotenoids, with peak enrichment in the $^{2}H_{10}$ species. Figure 4 (top) demonstrates the isotope profile of β-carotene from carrot grown hydroponically with 25 atom % $^{2}H_2O$ and analysed by LC/APCI-MS. The highest abundance peak is at *m/z* 547 [(M + 1) + 10]. In Fig. 4 (lower), the GC/ECNCI-MS analysis confirms that the labelled retinol formed from the labelled carrot dose has the most abundant enrichment peak at *m/z* 273 [(M + 1) + 5].

Spinach and carrots were harvested 32 and 60 days, respectively, after initiating the hydroponic growth in the $^{2}H_2O$-enriched medium. The spinach leaves (or carrots) were steamed in thin layers for 10 minutes. The cooked vegetables were immersed in cold water (1 litre water per 200 g vegetable) for 2 minutes, and then drained, pureed, sealed in a plastic container, and stored at -70°C before being used for the analysis of contents and for human consumption experiments.

Seven men (average age 56 years) each took the spinach and carrot in separate meals 3 months apart [74], to avoid possible interference between the doses, which were given in a random order. A fasting blood sample (10 ml) was drawn on day 0. Then, a liquid formula breakfast was given (25% energy from fat). In the middle of this meal, the subject took an oral dose of either spinach (300 g, thawed), or carrot (100 g, thawed). On day 7, the volunteer repeated the procedures described for day 0 of the study, except that he received as a reference dose a 3.0 mg [$^{2}H_8$]-retinyl acetate capsule together with a liquid formula meal. No vitamin supplements or large amounts of either β-carotene or vitamin A in the diet were permitted during this period. The process was repeated on day 90 with the other vegetable. The serum samples were analysed by GC/ECNCI-MS to determine the isotopic enrichment of retinol formed from the labelled vegetables. The enrichment of each isotopomer was counted in the calculation. The 300 g labelled spinach and 100 g labelled carrots each contained *ca.*11 mg (all-*E*)-β-carotene, and it was assumed that α-carotene and (*Z*)-β-carotene, which were also present, have half the activity of (all-*E*)-β-carotene. The retinol equivalences were determined to be 21 μg spinach β-carotene or 15 μg carrot β-carotene to 1 μg retinol.

With a similar approach, ten men (average age 48 years) each took 5 g dried *Spirulina* powder, containing 4.3 mg β-carotene [75]. When compared to a reference dose of 2.0 mg [$^{13}C_{10}$]-retinyl acetate in oil (capsule), 4.5 mg *Spirulina* β-carotene provided 1 mg retinol.

Another recent report demonstrated the absorption of β-carotene from intrinsically labelled kale and the formation of labelled retinol formed from the labelled kale β-carotene [76], but no conversion factor was estimated.

8. Conversion factors of β-carotene into retinol in humans: Summary

A summary of the major human studies to determine conversion factors for β-carotene, either synthetic or as a plant food constituent, into retinol is presented in Table 1. These data show that the conversion efficiency of vegetable β-carotene is very variable and poorer than previously thought.

Table 1. Summary of the results of studies to determine the conversion factor for β-carotene (β-C) in oil or in food sources. (n = number of subjects)

Food matrix	Method	Dose	Conv. factor	Ref
β-C in oil capsule n = 3	Depletion/repletion; adults	Repletion daily with 390 μg vitamin A (n = 1) or 1500 μg β-C (n = 2)	3.8 : 1	[41]
β-C in oil capsule n = 5	Depletion/repletion; adults	Repletion daily with 600 μg vitamin A (n = 2) or 1200 μg β-C (n = 3)	2 : 1	[42]
β-C and vitamin A in oil capsules n = 35	Enrichment plateau in school children (age 8-11) with normal or marginal vitamin A status	Twice daily, 80 μg [$^{13}C_{10}$]-β-C and 80 μg [$^{13}C_{10}$]-vitamin A in oil capsules for 21 days	1.5 or 2.4 : 1	[66]
β-C and vitamin A in oil capsule n = 1	Comparing AUC response to the β-C dose and the vitamin A reference dose in adults	β-C in oil, 6 mg β-C in oil, 126 mg	3.8 : 1 55 : 1	[68]
β-C and vitamin A in oil capsule n = 22	Comparing AUC response to the β-C dose and the vitamin A reference dose in adults	β-C in oil, 6 mg	9 : 1	[70,71]
β-C and vitamin A in oil capsule n = 1	Comparing AUC response to the β-C dose and the vitamin A reference dose in an adult	16.8 mg [$^{2}H_{6}$]-β-C in oil capsule and 10.2 mg [$^{2}H_{6}$]-vitamin A	15.9 : 1	[72]
Fruits, n = 49 Vegetables, n = 45 Retinol-rich foods, n = 48	Changes of serum retinol conc. in vitamin A-deficient (~0.7 μmole/L) anaemic school children (age 7-11)	Fruits: 509 RE/day Vegetables: 684 RE/day Vitamin A-rich foods: 556 RE/day	12 : 1 26 : 1	[52]

Table 1, continued.

Food matrix	Method	Dose	Conv. factor	Ref.
Green/yellow vegetables, n = 10 Light coloured vegetables, n = 8	Total body stores of vitamin A before and after the vegetable intervention in school children (age 5.3 -6.5) with normal or marginal vitamin A status	Green/yellow vegetables (206 mg calculated *E*-β-C) to prevent the decrease of 7.7 mg in liver stores	27 : 1	[54]
Sweet potato, Indian spinach, β-carotene capsule, or retinyl palmitate, (all, n = 14)	Mean changes of total body stores of vitamin A before and after a 60-day intervention in adult men compared with the mean changes in the retinyl palmitate group	Sweet potato, 750 μg RE Indian spinach, 750 μg RE β-carotene capsule, 750 μg RE retinyl palmitate, 750 μg RE	13 : 1 10 : 1 6 : 1	[55]
[^{2}H]-Labelled spinach, and vitamin A in oil capsule, n = 14	Comparing AUC responses to the spinach and the vitamin A reference dose in adults	Calculated 11 mg *E*-β-C from 300 g pureed, cooked spinach, and 3 mg [$^2H_8$]-vitamin A	21 : 1	[74]
[^{2}H]-Labelled carrot, and vitamin A in oil capsule, n = 7	Comparing AUC responses to the carrot and the vitamin A reference dose in adults	Calculated 11 mg *E*-β-C from 100 g pureed and cooked carrot, and 3 mg [$^2H_8$]-vit A	15 : 1	[74]
Fruit, n = 69 Leafy vegetables , n = 70 Retinol-rich foods, n = 70 Control, n = 68	Changes of serum retinol concentration in lactating women after taking fruit, vegetables or preformed vitamin A	Fruit, 4.8 mg *E*-β-C Vegetables, 5.6 mg *E*-β-C Retinol-rich diet, 610 μg retinol Control, 0.6 mg β-C and 1 μg retinol	12 : 1 28 : 1	[53]
Spirulina powder, n = 10	Comparing AUC responses to the *Spirulina* and the vit A reference dose in adults	4.3 mg *Spirulina E*-β-C	4.5 : 1	[75]

These findings illustrate that the vitamin A value of individual plant foods in humans is in need of further investigation. The β-carotene to vitamin A conversion factor is used as a guideline for dietary recommendations to aid in the fight to combat vitamin A deficiency worldwide, but there is wide variation between conversion factors reported in different studies and between individuals in a particular study. A value of at least 12:1 seems a more realistic guideline than the long-accepted 6:1.

D. Factors that Affect the Bioabsorption and Conversion *in vivo*

1. Vitamin A status

The efficacy of carotenoids as provitamin A is affected by vitamin A status. The activity of the intestinal β-carotene cleavage enzyme in vitamin A-sufficient rats is only half that in vitamin A-deficient rats [77]. Another study showed that the carotene cleavage is affected by the vitamin A concentration of the rats' diet [78]. Similar indications come from human studies *in vivo*. For example [79], after intervention with 40 g amaranth, children aged 2-6 years with initial serum retinol <25 μg/dL increased their serum retinol by 12.6 μg/dL, whilst those with initial serum retinol >25 μg/dL increased their serum retinol only by 6.2 μg/dL. In another study [51], children aged 7-12 years, with an average serum retinol concentration of 34 μg/dL, considered adequate, were given a β-carotene supplement (6 mg/day) or carrots containing 6 mg β-carotene per day. Neither intervention resulted in a change in the serum retinol concentration. These observations were further confirmed by a recent report [80] that, when provided with provitamin A carotenoids, children with inadequate vitamin A status (<25 μg/dL) showed the greatest increase in serum vitamin A concentration, whilst children with serum retinol >25 μg/dL showed very little or no response. As mentioned earlier, however, the change in serum retinol concentration before and after an intervention is not a good indicator, because the vitamin A formed from the supplement may contribute to increased body (liver) stores of vitamin A, but not to the serum retinol concentration of subjects with normal vitamin A status.

2. Food matrix

There are striking differences in the bioavailability of carotenoids and vitamin A from various food matrices [48,49]. The efficiency of absorption and uptake of β-carotene is discussed in *Chapter 7*. β-Carotene in spinach is present in protein complexes [81] located in chloroplasts. β-Carotene in carrots is largely in the form of carotene crystals in chromoplasts [81]. Different conversion factors have been observed for β-carotene from spinach and carrots. Several studies have shown that the carotene:retinol equivalency from fruits and vegetables is in the range of 12-27 μg of carotene to 1 μg of retinol [47,52]. These studies have shown that the food matrix affects the bioavailability of vitamin A and that carotenoids in fruit have better bioavailability than those in vegetables [14]. In the transgenic 'Golden Rice', β-carotene is in the yellow-coloured endosperm [82]. Rice endosperm contains starch and protein, and cooked rice is easy to digest. Thus, the efficiency of absorption and bioconversion of β-carotene from Golden Rice is predicted to be greater than that of β-carotene from spinach and carrot.

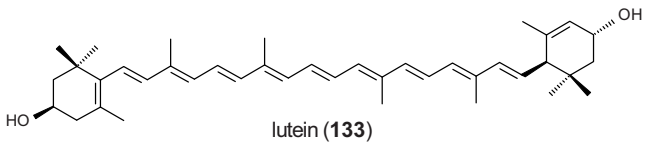
3. Food preparation

Food preparation practices have some effect on the bioavailability of carotenoids [83]. In a relevant study, subjects received, over a 3-week period, either a control diet (10 subjects), the control diet supplemented with β-carotene, or one of four spinach products (12 subjects per group): namely, (i) whole leaf spinach with an almost intact food matrix; (ii) minced spinach with the matrix partially disrupted; (iii) enzymically liquefied spinach in which the matrix was further disrupted, and (iv) liquefied spinach to which dietary fibre (10 g/kg wet weight) was added. Consumption of spinach in any of these forms significantly increased serum concentrations of (all-*E*)-β-carotene, (*Z*)-β-carotene and, consequently, total β-carotene and retinol. Serum total β-carotene responses, however, *i.e*. changes in serum concentrations of β-carotene from the start to the end of the intervention period, differed significantly between the groups fed whole leaf and liquefied spinach, and between the groups fed minced and liquefied spinach. Addition of dietary fibre to the liquefied spinach had no effect on serum carotenoid responses. The relative bioavailability of β-carotene from the spinach preparations compared with that of β-carotene from the carotenoid supplement was 5.1% for whole leaf spinach, 6.4% for minced spinach, 9.5% for liquefied spinach, and 9.3% for liquefied spinach plus added dietary fibre. Therefore, enzymic disruption of the matrix (cell wall structure) enhanced the bioavailability of β-carotene from whole leaf and minced spinach.

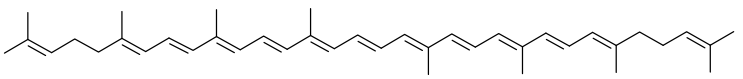
Another study reported that processing carrots as puree or by boiling and mashing can improve the bioavailability of carotenes and the vitamin A value [84].

4. Other carotenoids

It has been reported [85] that plasma β-carotene response is reduced in the presence of lutein (**133**), but no information was given on whether the conversion of β-carotene to retinol was also affected. Use of the postprandial chylomicron method to evaluate the effect of other carotenoids on the absorption and cleavage of β-carotene demonstrated that lutein, but not lycopene (**31**), led to a reduction in β-carotene absorption, though neither of these carotenoids affected the formation of retinyl palmitate [59].



lutein (**133**)



lycopene (**31**)

5. Protein malnutrition

The β-carotene 15,15′-oxygenase and 9,10-oxygenase enzymes have been found in intestine, liver, eye, and other tissues. Populations with protein malnutrition may, therefore, be deficient in these enzymes and will thus have diminished capability to convert β-carotene into vitamin A. In support of this, it has been reported that the activity of β-carotene cleavage enzymes in protein-deficient rats was significantly lower than in protein-adequate rats [86].

6. Intraluminal infections

It is common that populations at heightened risk of vitamin A deficiency are also likely to have a high prevalence of parasitic infestation and to rely on a high intake of plant food as provitamin A source. Data on whether parasitic infection affects vitamin A nutrition are somewhat conflicting [86]. The extent to which ascaris/hookworm infections affect the absorption of vitamin A and/or bioconversion of dietary provitamin A carotenoids to vitamin A remains to be determined.

7. Fat and fibre

The effects of fat content of a meal on the bioavailability of β-carotene have been investigated [87] (see *Chapter 7*). It has generally been accepted that a higher fat content in the diet facilitates the formation of intestinal micelles that are needed for absorption of vitamin A and carotene. A recent study [88] assessed the accumulation of β-carotene and vitamin A, derived from the β-carotene doses, in liver, kidney, and adrenal tissue of Mongolian gerbils that were given a β-carotene-deficient diet for 1 week, followed by one of eight isocaloric, semi-purified diets supplemented with carrot powder (1 μg β-carotene, 0.5 μg α-carotene/kJ diet) for 2 weeks (12 animals per group). Increasing dietary fat from 10% to 30% of total energy resulted in higher vitamin A tissue levels and lower β-carotene stores in the liver, suggesting that consumption of high-fat diets enhances conversion of β-carotene into vitamin A. Consumption of citrus pectin resulted in lower hepatic vitamin A stores and higher hepatic β-carotene stores compared with all other groups, suggesting lower conversion of β-carotene into vitamin A. In contrast, consumption of oat gum resulted in higher vitamin A and lower β-carotene stores in the liver, compared with values seen for gerbils fed citrus pectin. Further, the level of dietary fat consumed with soluble fibre had no interactive effects on hepatic vitamin A, β-carotene or α-carotene stores. These results demonstrate that absorption of β-carotene is affected independently by dietary fat level and type of soluble fibre, and suggest that these dietary components independently modulate the conversion of β-carotene into vitamin A.

A recent study [56] investigated how consumption of dietary fat at 7, 15, or 29 g/day with mixed vegetables containing 4.2 mg provitamin A per day affects total vitamin A pool size

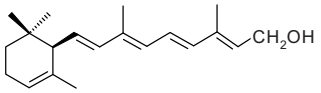
and the serum concentration of carotenoids. No difference was observed between groups taking various levels of dietary fat. Therefore, the requirement of dietary fat for optimal absorption of carotenoids appeared to be minimal.

E. Conversion in Tissues other than Intestine

Liver, fat, lung and kidney are capable of converting β-carotene into retinoids [31]. In addition to incubation studies with human and animal tissues, mathematical modelling has shown that, in order to fit a physiological compartmental model, the intestine and liver must be equally important in the conversion of β-carotene [63]. In the study in which 6 mg labelled β-carotene in corn oil was supplied to humans [70], the post-absorption conversion of β-carotene (the conversion of β-carotene after the intestinal absorption) *in vivo* was 7.8, 13.6, 16.4, and 19.0% at days 6, 14, 21, and 53, respectively.

F. Vitamin A Value of α-Carotene and (*cis*)-β-Carotenes

Other provitamin A carotenoids can also be converted into vitamin A *in vivo*. α-Retinol (*5*) was detected in livers of Mongolian gerbils fed α-carotene, and twice the amount of α-carotene than of β-carotene was needed to maintain vitamin A status in those gerbils [89]. In another study on gerbils, it was reported that the relative vitamin A values of (9*Z*)-β-carotene and (13*Z*)-β-carotene were 38% and 62%, respectively, of that of (all-*E*)-β-carotene [90]. The differences in the vitamin A value may be related to the intestinal absorption efficiency for the various isomers of β-carotene [91] or due to the different efficiencies of the isomers as substrates for the cleavage enzymes.



α-retinol (*5*)

G. Formation of Retinoic Acid from β-Carotene

Retinoic acid (*3*) plays an important role in the prevention and therapy of cancers, through its control of gene expression [92] (*Chapter 18*). β-Carotene can be converted into retinoic acid *via* an excentric cleavage pathway in ferret intestine [93,94] and in human intestinal mucosa [95]. The concentration of (all-*E*)-retinoic acid in the serum of rabbits fed β-carotene was found to be higher than in those fed no β-carotene [96]. Direct formation of retinoic acid from

β-carotene has been reported in hepatic stellate cells [97] and in rat intestine, kidney, liver, lung and testes [98]. A recent report, however, stated that the conversion of β-carotene into retinoic acid remains to be demonstrated in humans [99].

The pathway of formation of retinoic acid from β-carotene (*via* retinal or not), and the factors which affect the formation, warrant further investigation (see also *Chapter 18*).

H. Conclusion

Provitamin A carotenoids (mainly β-carotene) can provide vitamin A nutrition for humans. β-Carotene is converted enzymically into vitamin A in various tissues, and the small intestine is the prominent site for the conversion. The post-absorption conversion of absorbed β-carotene into vitamin A by tissues other than intestine is also likely and needs to be studied carefully.

The present reported values for β-carotene to vitamin A conversion show wide variation from 2 μg β-carotene:1 μg retinol for synthetic pure β-carotene in oil to 27 μg β-carotene:1 μg retinol for β-carotene from vegetables. Factors that affect β-carotene conversion to vitamin A include host nutrition status (vitamin A and protein nutrition), dietary fat and fibre content (macronutrient), food matrix (*e.g.* vegetables, fruits), and host intestinal health (parasitic infection and other infections). In an effort to increase the production of popular foods with better bioconversion factors, scientists are working to produce β-carotene-enriched staple foods through natural breeding and/or bioengineering techniques. Examples of such foods are Golden Rice, high β-carotene yellow maize, and high β-carotene ground nuts. These new food products will need rigorous scientific evaluation of their ability to provide vitamin A for combating vitamin A deficiency worldwide.

In human studies, the vitamin A value of pure β-carotene or of β-carotene in food can be determined quantitatively by using stable isotope techniques to study intrinsically labelled compounds and plants in conjunction with the paired DRD tests. It is not practical, however, to determine actual conversion factors for every population, individual or diet, in widely differing conditions. From the data that have been obtained in the various studies, it seems reasonable to think that a guideline conversion factor of at least 12:1 should ensure adequate provision of vitamin A from provitamin A carotenoids in food.

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