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CAROTENOID BIOAVAILABILITY AND BIOCONVERSION

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■ **Abstract** The possible role of carotenoids and their metabolites in disease prevention is far from fully understood, because the bioavailabilities of carotenoids are complicated by multiple factors that affect their absorption, breakdown, transport, and storage. Rapid progress in developing sophisticated methodologies, including use of stable-isotope dilution methods, now allows for an accurate determination of the true vitamin A activity of provitamin A carotenoids. The recent identification of specific enzymes, which catalyze the breakdown of β-carotene as well as nonprovitamin A carotenoids, is providing a better understanding of the functions of carotenoids at the molecular level. The pathways and possible mechanisms of carotenoid breakdown and factors affecting the bioavailability of carotenoids, such as carotenoid type, food matrix, interaction with other carotenoids and other food components, nutritional status, aging, and infection, are discussed in this review.

CONTENTS

INTRODUCTION

Carotenoids are lipid-soluble plant pigments found in photosynthetic plants and animal tissues. About 600 carotenoids have been isolated and characterized in nature, and about 10% of these can be metabolized to vitamin A in a variety of animal species. Both provitamin A carotenoids such as β- and α-carotenes and cryptoxanthins and nonprovitamin A carotenoids such as lutein, zeaxanthin, and lycopene are present in the blood and tissues of humans and have a variety of functions including free radical scavenging, enhancement of gap junction communication, and immunomodulation. Specific carotenoids may be responsible for different health promoting effects. Hydrocarbon carotenoids such as β-carotene and lycopene, which are abundant in yellow and orange colored fruit and vegetables, are related to a reduced risk of site specific cancers and heart disease (2, 20, 34, 35, 42, 70, 92, 110, 132, 133), whereas oxygenated carotenoids such as lutein and zeaxanthin, which are abundant in dark-green leafy vegetables, may be important for protecting eye tissues (44, 67).

A possible role for carotenoids in disease prevention beyond the known function as a vitamin A precursor has stimulated interest in carotenoid absorption and metabolism. However, these topics are far from fully understood, because the bioavailabilities of carotenoids are complicated by multiple factors that affect their absorption, breakdown, transport, and storage. The recent development of sophisticated laboratory methodologies and the recent identification of specific enzymes that catalyze the breakdown of β-carotene and nonprovitamin A carotenoids is allowing us to gain a better understanding of the functions of carotenoids at the molecular level.

ABSORPTION AND TRANSPORT OF CAROTENOIDS

Comprehensive reviews of carotenoid absorption and metabolism have been published by Erdman et al. (31), Parker and colleagues (84, 85, 87), Castenmiller & West (15), and van Vliet (117). After consumption of carotenoid-containing foods, carotenoids are released from their food matrix and incorporated into mixed micelles, which consist of bile acids, free fatty acids, monoglycerides, and phospholipids. The amount of carotenoid incorporated into micelles depends on the polarity of the carotenoid and on micellar fatty acid composition and saturation. Carotenoids appear to be absorbed by the mucosa of the small intestine (mainly in the duodenum) via passive diffusion (50, 84) to become packaged into triacylglycerol-rich chylomicrons. Therefore, release of carotenoids from the food matrix and dissolution in the lipid phase are critical steps in the absorption process.

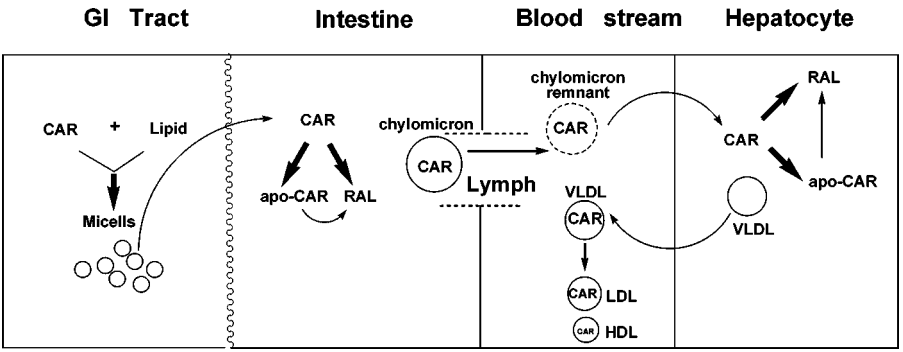

Figure 1 Absorption, metabolism, and transport of carotenoids. CAR, carotenoids; apo-CAR, apo-carotenoids; RAL, retinal; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

Provitamin A carotenoids, such as β-carotene, α-carotene and cryptoxanthin, are partly converted to vitamin A, primarily retinyl esters, in the intestinal mucosa, and both carotenoids and retinyl esters are incorporated into chylomicrons and secreted into lymph for delivery to the blood stream, where the chylomicrons are rapidly degraded by lipoprotein lipase. The resulting chylomicron remnants containing carotenoids are rapidly taken up by the liver (84). The liver secretes carotenoids associated with hepatic very low density lipoprotein (VLDL), but in the fasting state most plasma carotenoids are associated with low density lipoproteins (LDL) and high density lipoproteins (HDL). Chylomicron carotenoid levels peak early (4–8 h) after ingestion of carotenoids owing to intestinal excretion, whereas LDL carotenoid levels in the circulation peak at 24–48 h, and HDL levels peak at 16–48 h (19). In fasting blood up to 75% of hydrocarbon carotenoids such as β-carotene and lycopene are found in LDL, and the remaining carotenoids are associated with HDL and to lesser degree with VLDL (19, 31, 59, 66). The more polar carotenoids such as lutein and zeaxanthin are evenly distributed between LDL and HDL fractions in fasting blood (Figure 1).

Lipophilic carotenoids are mainly located in the core of lipoprotein, which may not allow their transfer between lipoproteins at an appreciable rate (75), whereas the more polar carotenoids, which are mainly present on the surface of lipoproteins, are likely to undergo rapid surface transfer, resulting in a more equal equilibration between LDL and HDL.

Nonprovitamin A carotenoids (e.g., lutein, zeaxanthin, lycopene) are absorbed intact (85), although oxidative cleavage of nonprovitamin A carotenoids could occur to some extent before absorption from the intestinal lumen. Oxidative cleavage products of lutein, zeaxanthin, and lycopene in human milk and serum have all been reported (62). Fatty acid esters of oxygenated carotenoids (lutein and zeaxanthin) are cleaved in the lumen of the small intestine prior to uptake by the mucosa, most likely by carboxylic ester hydrolase secreted by the pancreas (71).

Carotenoid concentrations vary substantially from tissue to tissue (60, 98, 103). Tissues that have a large number of LDL receptors (liver, fat) probably accumulate carotenoids passively. However, the variable concentrations and forms of carotenoids in different tissues suggest that other factors play a role in uptake and accumulation of carotenoids in tissues. For example, the macular pigments of the eye are primarily lutein and zeaxanthin (4, 6, 7), suggesting the presence of a binding protein.

METABOLISM OF CAROTENOIDS

Absorbed β-carotene, and presumably the other provitamin A carotenoids, can undergo oxidative cleavage in intestine as well as in other organs such as the liver. As shown in Figure 2, various mechanisms of breakdown of carotenoids have been suggested by the identification of several oxidative products of carotenoids upon their incubation (64, 77) with either tissue homogenates (124) or the postmitochondrial fraction (130) or cytosol (28, 29) of a variety of tissues from animals or humans, and by identifying the carotenoid-cleaving enzymes at the molecular level (63, 122).

The eventual elucidation of the actual contribution of provitamin A carotenoids to vitamin A and the biological function of carotenoids and their metabolites will allow for more accurate recommendations for dietary intakes of these carotenoids. Although there is no Recommended Dietary Allowance for carotenoids, the Dietary

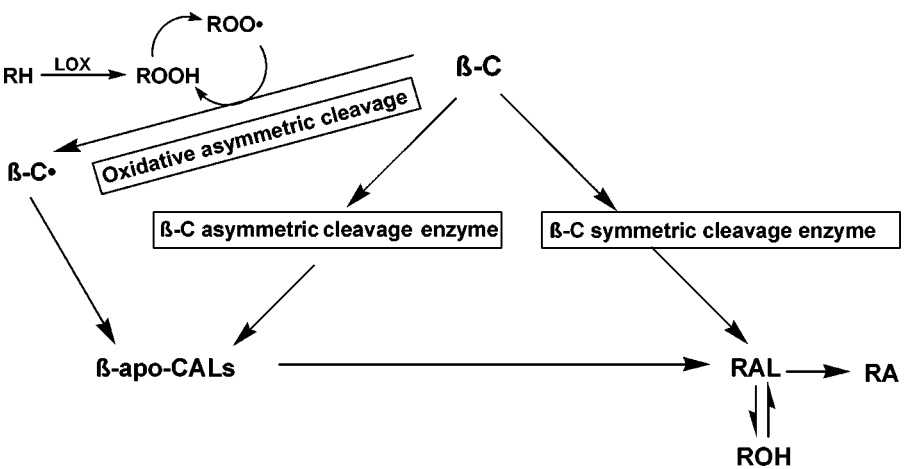

Figure 2 Possible pathways of oxidative cleavage of β-carotene. β-C, β-carotene; β-C•, β-carotene radical; β-apo-CALs, β-apo-carotenals; RAL, retinal; RA, retinoic acid; ROH, retinol; LOX, lipoxygenase; RH, polyunsaturated fatty acid; ROOH, hydroperoxide; ROO•, hydroperoxyl radical.

Reference Intake Committee recently changed the dietary conversion factors from 6 μg β-carotene or 12 μg of other provitamin A carotenoids being equal to 1 μg retinol (retinol equivalent) to 12 μg β-carotene or 24 μg of other provitamin A carotenoids being equal to 1 μg retinol (retinol activity equivalent). Dietary carotenoids from food are more poorly absorbed than pure carotenoids (32). For example the conversion factor used for β-carotene in oil is 2 μg β-carotene equals to 1 μg retinol.

Pathways of β-Carotene Cleavage

The central cleavage mechanism splits β-carotene at the central double bond (15,15′) by a specific enzyme, β-carotene 15,15′-oxygenase (E.C.888990988), found to yield retinal in intestinal cell and liver cytosol (41). The cleavage product, retinaldehyde, can be reversibly reduced to retinol (vitamin A) or irreversibly oxidized to retinoic acid (81) (see Figure 2). Several in vitro studies have shown almost exclusive central cleavage in the intestines of guinea pig (28), pig (88), rat, and hamster (22, 77, 119). An in vivo study using rats (1) has shown the same. Therefore, central enzymatic cleavage of β-carotene has an essential role to provide vertebrates with vitamin A.

A new era in vitamin A research was opened up by German scientists who successfully cloned and sequenced cDNAs encoding enzymes having β-carotene 15,15′-oxygenase activity from drosophila (122) and chicken duodenal tissue (126, 127). Further, it has been suggested that the central cleavage enzyme is not a dioxygenase (as previously thought) but a monooxygenase (68). In this sophisticated work the nonsymmetrical carotenoid, α-carotene, was used as a substrate for the central cleavage enzyme that had been purified from chicken intestinal mucosa to yield different aldehydes (126). This work demonstrated the incorporation of oxygen from water and/or air into cleavage products via a monooxygenase mechanism: epoxidation of the central double bond of α-carotene followed by unselective ring opening with water and final diol cleavage to yield the aldehydes.

An additional random cleavage process for carotenoids was first proposed by Glover & Redfearn (38), who observed cleavage at several double bonds in the polyene chain of β-carotene in addition to the central 15,15′-double bond to produce β-apo-carotenals, which can be subsequently converted to retinal. Random cleavage of β-carotene was supported by the identification of β-apo-carotenoids in the intestine of chickens after β-carotene supplementation (99). Furthermore, Wang et al. (124) demonstrated the formation of β-apo-carotenals from in vitro incubations of β-carotene with the postnuclear fraction of intestinal tissues from humans, monkeys, ferrets, and rats, as well as from in vivo studies using ferrets (123).

Kiefer and colleagues (63) identified an enzyme that exclusively catalyzes the asymmetric oxidative cleavage of β-carotene at the 9′,10′ double bond of β-carotene, resulting in the formation of β-apo-10′-carotenal and β-ionone. Dmitrovskii and colleagues (25) also reported an enzyme, which is different from

β-carotene-15,15′-oxygenase, involved in the enzymatic oxidation of β-apo-8′-carotenol to β-apo-14′-carotenal.

Carotenoids also can be broken down by free radicals produced by enzymes such as lipoxygenase. In a recent study Gessler and colleagues (37) reported that free radical oxidation of arachidonic acid with lipoxygenase inhibited the central cleavage of β-carotene. Moreover, a lipoxygenase inhibitor and antioxidants promoted conversion of β-carotene into retinal. In accordance with this study, it has been reported that random cleavage products of β-carotene can be formed by lipoxygenase in the presence of substrate (linoleic acid) or by linoleic acid hydroperoxide (130). In further work, Yeum and colleagues (129) demonstrated that both central and random cleavage of β-carotene can take place in the post-mitochondrial fraction of rat intestine, but that the pathway depends on the presence or absence of the antioxidant α-tocopherol. In their work on the presence of α-tocopherol, central cleavage mainly occurred (i.e., β-carotene was converted to retinal), whereas in the absence of α-tocopherol both random cleavage and central cleavage took place (i.e., both retinal and β-capo-carotenals were produced). In recent work of Caris-Veyrat et al. (14) mild oxidative cleavage of β-carotene by dioxygen was induced by a ruthenium tetramesitylporphyrin catalyst. This led to the full range of β-apo-carotenals and β-apo-carotenones, indicating the involvement of free radicals to produce random cleavage products, i.e., β-apo-carotenals. This supports earlier studies that demonstrated the formation of β-apo-carotenals from β-carotene by radical attack (13, 45) or singlet oxygen (105), although in these in vitro systems the partial presence of oxygen and the concentration of β-carotene were very high (13, 45). Gomboeva and colleagues (39) reported the activity of β-carotene 15,15′-oxygenase to be decreased in the presence of oxidant but protected by antioxidants, thus providing more evidence for an important role of antioxidants in promoting central cleavage of β-carotene.

In summary, it appears that β-apo-carotenals with different carbon chain lengths can be produced by enzymatic reactions (63), cooxidation by lipoxygenase (37, 130), autoxidation, or direct reaction with free radicals (45, 69, 76), and that the mechanism of breakdown depends on the conditions in which the experiment has been run. The molecular identification of the enzymes involved in the cleavage of β-carotene and the use of modern techniques for studying the reaction mechanisms of β-carotene metabolism have produced new insights into the metabolism of β-carotene. Considering that the stoichiometry of retinal production per mole of β-carotene is 1.72–2.00 mol (22, 129) and that the total amount of the β-apo-carotenoids is $<5\%$ of the retinoids formed in the intestine from β-carotene (1), it is certain that the enzymatic central cleavage of β-carotene plays the major role in β-carotene breakdown under normal conditions when an adequate supply of antioxidants is available. However, under conditions of oxidative stress (such as smoking or diseases associated with oxidative stress such as cancer) or when high β-carotene concentrations are present, both enzyme-related and radical-induced random cleavage can play a role in β-carotene breakdown. The species and tissue specificities of β-carotene cleavage and the factors controlling cleavage activity remain to be elucidated.

Breakdown of Other Carotenoids

Lycopene, a nonprovitamin A carotenoid, has received much attention for its potential beneficial effect on human health. Owing to its chemical structure, which has two more double bonds than β-carotene, lycopene exhibits a physical quenching rate constant with singlet oxygen that is almost twice as high as that of β-carotene (100). The oxidation of lycopene can occur by photosensitized oxidation (111) or by exposure to hydrogen peroxide (72). Khachik and colleagues (61) found an oxidation product of lycopene, 5,6-dihydroxy-5,6-dihydrolycopene, in human serum. Further, Kim and colleagues (64) recently reported autoxidation of lycopene to produce two acycloretinoids (acycloretinal and acycloretinoic acid) as well as a series of apo-lycopenals (14′,12′,10′,8′, and 6′) by incubating lycopene solubilized in either 50 μM toluene, aqueous Tween 40, or a liposomal suspension at 37°C for 72 h. On the other hand, Kiefer and colleagues (63) reported that lycopene can also be cleaved by the same enzyme that specifically cleaves the 9′,10′ double bond of β-carotene. This indicates in respect to substrate specificity that the polyene chain backbone of carotenes plays an important role, whereas the ionone ring structures of β-carotene seem to be of marginal relevance. One could speculate that this enzyme is also able to catalyze the oxidative cleavage of other carotenoids in addition to β-carotene and lycopene.

Canthaxanthin also yielded a series of cleavage products such as 4-oxo-β-apocarotenals upon oxidation with nickel peroxide (73). Another oxidation product of canthaxanthin is 4-oxo-retinoic acid, which activates the retinoic acid receptor gene promoter to enhance gap junctional communication (47, 78, 101).

FACTORS AFFECTING THE BIOAVAILABILITY OF CAROTENOIDS

As mentioned earlier, the bioavailability of carotenoids from either food or pure synthetic products is quite variable, because the release from the food matrix, lipid micelle formation, uptake of carotenoids into intestinal mucosal cells, and transport of carotenoids and their metabolic products are all affected by a complex set of factors.

Carotenoid Type

The bioavailability of hydrocarbon carotenoids such as β-carotene is relatively lower than that of oxygenated carotenoids such as lutein and zeaxanthin. Owing to their more polar nature, oxygenated carotenoids can more easily be incorporated into the outer portions of lipid micelles within the gastrointestinal tract and therefore can be more easily taken up by enterocyte membranes and eventually chylomicrons, hence increasing their bioavailability. This is supported by the work of van het Hof et al. (114), who showed that the absorbability of lutein from vegetables was five times higher than that of β-carotene. The high relative absorbability

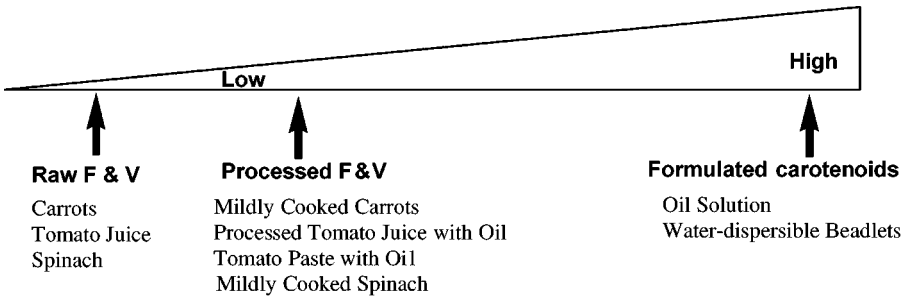

Figure 3 Bioavailability of carotenoids as a function of food matrix. F & V, fruits and vegetables.

of lutein compared with that of β-carotene is also in line with the findings of Johnson et al. (55). In their study, seven healthy adults modified their daily diets by adding 60 g/day of spinach (10.8 mg/day lutein, 5 mg/day β-carotene) for 15 weeks. The serum lutein concentrations increased twofold from baseline values ($p < 0.001$) after 4 weeks of spinach feeding and remained higher than baseline throughout the 15-week supplementation period, whereas the mean serum concentrations of β-carotene did not change throughout the entire study period. Thus, the absorbability of lutein is higher than that of β-carotene and/or lutein could interfere with the absorption of β-carotene.

Food Matrix

In nature carotenoids in a wide variety of plants, animals, and microorganisms are found complexed with protein. Therefore, release from the food matrix is an important initial step in the absorption process. It has been suggested that carotenoproteins have an inhibitory effect upon carotenoid digestion and absorption (12, 23). Studies on the effect of food matrix on carotenoid bioavailability have generally compared the responses of the pure formulated, natural, or synthetic carotenoid with the equivalent carotenoid dose found in a food source. Several investigators have found that pure β-carotene dissolved in oil or aqueous dispersions is efficiently absorbed (>50%) (3, 40, 95), whereas carotenoids in uncooked vegetables such as β-carotene in the carrot (95) or lycopene in tomato juice (104) are poorly absorbed (<3%) (Figure 3).

The effect of food matrix on the bioavailability of carotenoids affects the outcome of intervention trials using provitamin A carotenoids from food sources to improve vitamin A status. For example, daily supplementation of dark-green leafy vegetables for 12 weeks to lactating Indonesian women with low vitamin A status did not increase plasma vitamin A level, whereas a similar amount of β-carotene (3.5 mg) given in a wafer supplement led to a significant increase in plasma retinol (21).

FOOD PROCESSING The effect of food processing on carotenoid bioavailability can be illustrated by comparing the blood responses after eating a raw food compared with food that has been heat-treated and/or mechanically homogenized to disrupt the food matrix (11). Castenmiller and colleagues (16) examined serum carotenoid responses after chronic consumption (3 weeks) of variously processed spinach products: whole leaf, minced, and enzymatically liquefied forms. Serum concentrations of β-carotene and lutein were significantly increased by spinach consumption in any form. However, in contrast to the serum total β-carotene responses, which were significantly increased by ingesting minced spinach (increased from 5.1% to 6.4% compared with that of pure β-carotene supplement) and further increased to 9.5% by consumption of liquefied spinach, the lutein response did not differ among the three spinach-fed groups (45%, 52%, 54%, respectively). It is probable that plasma lutein levels had already reached a constant elevated plateau level by ingesting whole leaf of spinach for 3 weeks. In contrast, van het Hof and colleagues (116) showed significantly higher plasma responses of lutein after ingestion of chopped spinach than after ingestion of whole leaf spinach for 4 days. Chopping did not, however, affect the plasma response to β-carotene during these 4 days of ingestion of spinach. In a two 4-week cross-over feeding study (93) the plasma response of β-carotene was three times greater after feeding spinach and carrots that were pureed and thermally processed than when these vegetables were consumed raw. Dietz and colleagues (23) reported an increase of extractability of carotenoids in carrots by steaming. However, more prolonged exposure to high temperatures (boiling) reduced the bioavailability of carotenoids by increasing the oxidation and production of more isomers.

Similar to the findings on β-carotene bioavailability, feeding heat-treated tomatoes was associated with a greater increase in serum (104) or triglyceride-rich lipoprotein (TRL) (33) lycopene than feeding unprocessed tomato juice. The improvement of bioavailability of lycopene by mechanical homogenization and heat treatment was confirmed by van het Hof et al. (115). In their study lycopene absorbability was significantly enhanced by tomato homogenization and tended to be enhanced by heating (1 h at 100°C) as measured by the lycopene response of the TRL fraction of plasma after a single dose of tomato or after 4 days of tomato consumption (22 mg/day lycopene).

ISOMERIC FORMS Several different geometric isomers of β-carotene (all-*trans*, 9-*cis*, 13-*cis*, and 15-*cis* isomeric forms) exist in food and human tissues (17). The major β-carotene isomer in the circulation of humans is all-*trans*-β-carotene, with small amounts of 13-*cis* and 9-*cis* β-carotene. However, circulating levels of the *cis* isomers of β-carotene are not responsive to increased consumption of their isomers (102, 103, 106). Data examining the serum response to a single large oral dose of either all-*trans*-β-carotene or 9-*cis* β-carotene in men indicated that the all-*trans* isomer attains a far greater postprandial concentration (36, 56). In an attempt to determine if these serum response differences were due to differences in tissue uptake, the concentration of β-carotene isomers in human serum, breast milk, and

buccal mucosa cells after continuous oral doses of β-carotene isomers (all-*trans* and 9-*cis*) were examined in healthy lactating women (57). The changes of all-*trans* and 9-*cis* β-carotene in milk and buccal mucosa cells followed a pattern similar to that for serum, suggesting that the differences in the serum response of all-*trans* β-carotene vs 9-*cis* β-carotene reflect selective intestinal absorption of the all-*trans* β-carotene or conversion of 9-*cis* β-carotene to all-*trans* β-carotene. In fact, You et al.'s work using stable isotopes (131) indicates that substantial proportions of oral doses of 9-*cis* β-carotene can undergo isomerization to all-*trans* β-carotene between ingestion and appearance in plasma.

In contrast to the food sources in which all-*trans* lycopene comprises 79–91% of total lycopene, *cis* forms of lycopene make up $>50\%$ of the total lycopene in human serum and tissues (18, 103). It has been suggested that *cis*-isomers of lycopene are better absorbed than all-*trans* form (11, 103). Holloway et al.'s finding (51) that plasma contains only 40–45% all-*trans* lycopene after consuming cooked tomato puree (84% all-*trans*) indicates either isomerization of lycopene or better absorption of *cis* isomers—or both. A recent study by Re et al. (90) demonstrated isomerization of all-*trans* lycopene to *cis*-isomers in the acidic environment of the stomach. The bioavailability of isomers of other carotenoids and the importance of these isomeric forms to human health and disease remain to be explored.

Interaction with Other Carotenoids

Carotenoid interactions have been studied between β-carotene and oxygenated carotenoids such as lutein, as well as between β-carotene and lycopene. van den Berg (112) has extensively reviewed this subject.

Several studies provide direct evidence for differential carotenoid absorption in humans. Kostic et al. (65) examined serum responses as area under the curve (AUC, i.e., the area under the serum response curve) after single doses of β-carotene and lutein, both alone and after an equimolar mixture (0.5 μmol/kg body weight for each). They found that when combined, β-carotene significantly reduced the serum responses for lutein to 53–61% of control values, suggesting the interaction between these two carotenoids. The reduced absorption of lutein by β-carotene was supported by O'Neill and Thurnham (82), who examined intestinal absorption of β-carotene, lutein, and lycopene using the response curves in the TRL fraction after a single oral dose of 40 mg of β-carotene taken with either 31.2 mg of lutein or 38 mg of lycopene. The estimated absorptions (determined by AUC) were similar for β-carotene and lycopene but were significantly lower for lutein. β-carotene also appears to reduce the absorption of canthaxanthin (83). Combined doses of β-carotene and canthaxanthin (25 mg each) resulted in plasma canthaxanthin responses, which are significantly lower than after canthaxanthin alone. However, canthaxanthin did not inhibit the β-carotene appearance.

It should be noted that provitamin A carotenoid cleavage is not accounted for by proxy measurements of β-carotene absorption; thus, absorption of β-carortene has been generally underestimated. van den Berg & van Vliet (113) took into account the cleavage of β-carotene to examine the relative bioavailability of β-carotene

compared with lutein and lycopene. The combined dosages of β-carotene (15 mg) with lutein (15 mg) decreased the AUC of β-carotene and retinyl palmitate in the TRL fraction by 66% and 74%, respectively, compared with the response to β-carotene alone. On the other hand, a combined dosage of β-carotene with lycopene (15 mg) had no significant effect on the β-carotene or retinyl palmitate responses. Neither lutein nor lycopene affected the β-carotene cleavage, as estimated by the ratio between the AUC for retinyl esters and the AUC for β-carotene.

The differential absorption and/or interactions between carotenoids can also be seen in controlled feeding studies with diets or foods containing carotenoids. Martini and colleagues (74) found a higher ratio of β-carotene to α-carotene in the diet (3.9) than in plasma (1.8) of volunteers fed a low-carotenoid control diet with extra carrots and spinach. Also, Yeum et al. (128) showed high serum responses for α-carotene and cryptoxanthin, but low serum responses for lutein relative to the dietary intake, which supports selective absorption of carotenoids.

Carotenoids can interact with each other at any point during the absorption, metabolism, and transport process. In the intestinal mucosa, carotenoids may inhibit or enhance the activity of carotenoid cleavage enzymes. In the circulation, there may be exchange of carotenoids among plasma lipoproteins, which could be affected by the type and amount of carotenoid present. There may also be inhibition or enhancement of tissue uptake and/or release of one carotenoid by another.

Interaction with Fat and Fiber

Evidence has accumulated that in order to optimize carotenoid absorption (83), dietary fat needs to be consumed during the same eating period as the carotenoid. The absorption and bioconversion of β-carotene is significantly reduced when the intake of dietary fat is not adequate (54, 88). Dimitrov and colleagues (24) showed that dietary fat increases the plasma response to β-carotene supplements, although the amount of fat required for optimal absorption of vegetable carotenoids may be quite small (53). Roodenburg and colleagues (96) found that the amount of dietary fat consumed (3 or 36 g) did not affect increases in plasma concentrations of α- and β-carotene after α- and β-carotene supplementation (8 mg) but did result in an increase in the plasma concentration of lutein (88% vs. 207% increase) after lutein ester supplementation (8 mg lutein), which indicates that the amount of fat required for optimal intestinal uptake of specific carotenoids may be different.

It is possible that dietary fiber decreases the bioavailability of carotenoids by entrapping carotenoids and by interacting with bile acids resulting in increased fecal excretion of fats and fat-soluble substances such as carotenoids. Rock & Swendseid (94) examined the effect of dietary fiber (12 g of citrus pectin) on the serum β-carotene response. The increase in plasma β-carotene concentration after ingestion of β-carotene in a capsule (25 mg) was significantly reduced by pectin (42%). Hoffmann and colleagues' (48) study, in which dietary fiber, pectin, guar, or cellulose supplementation decreased the antioxidant effect of a carotenoid and α-tocopherol mixture, also suggests the reduced absorbability of these nutrients by fiber.

Nutritional Status

In 1961 Olson (80) first reported that injection of vitamin A together with [^{14}C]-β-carotene into ligated loops of rat intestine inhibited the formation of [^{14}C]-retinyl ester. Since then, animal studies showed that bioconversion of β-carotene to vitamin A was decreased by an increased intake of vitamin A (26, 120, 121). In contrast, Gronowska-Senger & Wolf (43) reported that depletion or excess feeding of β-carotene or retinol did not greatly affect the intestinal β-carotene 15,15′-oxygenase activity in rats. Recently Ribaya-Mercado et al. (91) showed that the improvement of vitamin A status after a dietary intervention is strongly influenced by total body stores of vitamin A measured using a 3-day deuterated retinol isotope-dilution procedure with deuterated vitamin A. However, the effect of nutritional status on the bioavailability and bioconversion of β-carotene is still not clear in humans.

Aging

The greatest change in gastrointestinal physiology affecting nutrient bioavailability that has been identified with advancing age is atrophic gastritis, which occurs in a large percentage ($\sim$20%) of the elderly population and results in reduced stomach acidity. Atrophic gastritis appears to affect the bioavailability of carotenoids, the absorption of which is pH dependent (97), because the pH in the proximal intestinal lumen can affect the surface charges of both the micellar particles and the luminal cell membrane, with less diffusion resistance at a lower pH (49). Tang and colleagues (109) reported that inhibition of gastric acidity decreases the blood response to β-carotene, thereby implicating a negative effect of atrophic gastritis on β-carotene absorption.

Parasite Infection

The subject of whether parasite infection affects the bioavailability of carotenoids is controversial. Jalal and colleagues (52) conducted a study to examine the effect of food sources of β-carotene, dietary fat, and *Ascaris lumbricoides* infection on serum retinol concentration in children (3–6 years) for 3 weeks. The greatest rise in serum retinol occurred when meals contained added β-carotene sources and added fat and when the children were dewormed. The effects of fat and deworming together were additive to the effects of additional β-carotene sources.

METHODS TO DETERMINE BIOAVAILABILITY OF CAROTENOIDS

Currently there are no validated methods for the quantitative assessment of bioavailability of carotenoids from dietary sources or synthetic supplements. However, rapid progress in the application of a stable-isotope dilution method to determine the bioavailability of carotenoids has begun a new era for understanding the true vitamin A activity of dietary provitamin A carotenoids.

Serum/Plasma Response After Carotenoid Ingestion

Plasma or serum carotenoid responses (concentration vs. time curves) have been widely used to measure carotenoid bioavailability, because this method provides an estimate of relative bioavailabilities using simple procedures. In this method quantitated amounts of carotenoids are ingested and changes in serum concentration of carotenoids are measured at various time intervals following ingestion. Comparisons of relative bioavailabilities can be made between different carotenoids, formulations (e.g., purified vs. food), food preparations (e.g., processed vs unprocessed food), or individuals. Serum response curves are carried out using either single or multiple doses. A rise in serum concentration followed by a fall is generally measured. However, in chronic dose trials, serum carotenoid concentrations reach a constant elevated plateau level of various magnitudes.

Serum response curves to determine bioavailability are limited by several factors: (*a*) The serum response to a single oral dose of carotenoid is highly variable (58); (*b*) the concentration of carotenoid in serum represents a balance between intestinal absorption, breakdown, tissue uptake, and release from body stores; (*c*) human serum contains substantial endogenous concentrations of carotenoids such as α-carotene, β-carotene, lycopene, cryptoxanthins, and lutein; and (*d*) provitamin A carotenoids can be metabolized to retinyl esters during intestinal absorption. For these reasons, relatively large doses, usually exceeding the typical daily intake by at least fivefold, are needed for a significant increase in carotenoid concentrations over baseline levels. However, it is probable that large doses overwhelm transport and metabolic processes, or at least alter rate constants of metabolism or transport, thereby making interpretation of results difficult.

Chylomicron Response after Carotenoid Ingestion

Carotenoid concentrations in triglyceride-rich lipoprotein (TRL) fractions (mixtures of chylomicrons and VLDLs) have also been used to estimate between-person as well as within-person variability in β-carotene absorption and intestinal conversion to retinyl esters (118).

Advantages of this method over the serum response curve method are that (*a*) the method accounts for intestinal conversion to retinyl esters; (*b*) it improves the distinguishability of newly absorbed carotenoids from endogenous pools; and (*c*) it allows for the use of smaller doses. However, this method is not able to separate the liver-derived VLDL from the intestine-derived chylomicrons. Also, use of this relative bioavailability measurement may be problematic with oxygenate carotenoids, which are susceptible to rapid surface transfer to LDL or HDL, as discussed above. A potential limitation of this approach is that food matrices that are slowly digested result in slow rates of carotenoid absorption and thus yield little or no rise of carotenoids in the TRL fraction.

As observed with serum response curves, TRL response curves are highly variable (113, 118), especially among subjects, even when treatment conditions are highly standardized. This may be due to variability in carotenoid absorption as well as in the kinetics of chylomicron secretion and clearance.

Oral-Fecal Balance Technique

Comparison of carotenoid consumption with its fecal excretion (i.e., balance) has been used for the estimation of absorption of carotenoids, particularly from foods. Balance studies involve the estimation of carotenoid intake and the collection and analysis of all feces for carotenoids over a period of time, because there is no urinary excretion of either free or conjugated carotenoids and there is negligible loss with exfoliation from skin. The balance method has major limitations: It does not account for (*a*) carotenoid degradation in the upper (chemical oxidation) or lower (microbial degradation or alteration) regions of the gastrointestinal tract or (*b*) the excretion of endogenously secreted carotenoids. Therefore, it is not surprising that oral-fecal balance studies have yielded considerable variation in estimates of carotenoid absorption, even with seemingly similar carotenoid sources or preparations. In an attempt to overcome this limitation, Bowen et al. (10) modified the method by using gastrointestinal lavage (washout) after allowing a defined period for digestion and absorption. The advantage of this approach is that it controls the residence time of nonabsorbed carotenoids in the lower gut, thus limiting microfloral degradation. However, the duration of the allowed absorption period in this approach is arbitrary and it may alter gastrointestinal physiology.

Stable Isotope Application

The development of stable isotope labeled carotenoids has made it possible to (*a*) distinguish between dosed and endogenous carotenoids, (*b*) assess the extent of intestinal conversion of vitamin A, (*c*) estimate absolute absorption and postabsorptive metabolism for subsequent empirical or compartmental modeling, and (*d*) use doses that are low enough to avoid influencing endogenous pools (79). To date, the published research regarding use of isotopic applications to determine carotenoid bioavailabilities have focused on β-carotene. The stable isotope method reported by Dueker el al. included the isolation and quantification of all-trans octadeuterated β-carotene (β-carotene-d8) and retinol-d4 derived from β-carotene-d8 following an oral dose and using reverse-phase high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) with electron ionization (27). Parker et al. reported a stable isotope tracer method, which they successfully employed in a human study using oral doses of ^{13}C-labeled β-carotene as low as 0.5 mg and a high precision gas isotope ratio mass spectrometer (86).

In these methods single doses of deuterated or ^{13}C-enriched β-carotene are administered to subjects under standardized conditions. Serial blood samples are drawn at baseline frequently over the first 16 h, then less frequently if postabsorption data are wanted. Because the absorption (chylomicron) peak typically occurs at 4–5 h after dosing, frequent sampling (at least hourly) is needed during this period to obtain accurate AUC kinetic parameters. Owing to the extensive fraction purification required, these methods are labor intensive and costly, which limits sample size.

A stable-isotope reference method has been developed for the quantification of bioavailability and bioconversion of β-carotene to retinol in humans using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (APCI LS-MS) (107, 125). Following oral administration of ^{13}C-retinyl palmitate and ^{13}C-β-carotene[10] at physiologic doses, blood samples are collected and analyzed using an HPLC interfaced to an APCI LS-MS. This method appears to be able to study retinol equivalency of β-carotene. Tang et al. (108) applied this method to determine the bioavailability of β-carotene in spinach. They gave a single dose of 3 mg $^2H_8$ retinyl acetate and 200 g spinach (13 mg of ^{2}H-labeled β-carotene, 2.7 mg of deuterated other provitamin A carotenoids) grown on 30% ^{2}H-labeled H_2O to two adults and found that the vitamin A activity of 50 μg β-carotene in spinach was equivalent to that of 1 μg retinol. A similar method using $^2H_4$ retinyl acetate as an extrinsic reference standard to determine the bioavailability of provitamin A carotenoid in plant foods has been reported, although unlabeled vegetables (carrot or spinach) were used in this study (30). In this method concentrations of unlabeled and labeled retinyl esters and carotenoids in the plasma TRL fraction were determined in serial blood samples with HPLC and GC/MS.

Stable isotope labeling approaches appear to be the best approach for studying carotenoid bioavailability from pure formulas or foods, because the bioavailability of carotenoids within a food matrix can be obtained when given at a physiologic dose, and by using a reference dose the vitamin A equivalency of carotenoids can be determined.

Macular Pigment Density Measurement

The oxygenated carotenoids, lutein and zeaxanthin, are a major macular pigment of the human retina. Macular pigment density, which can be assessed noninvasively by a psychophysical method to assess its relationship with carotenoid intake, may be a functional indicator of the bioavailability of lutein or zeaxanthin (4, 5, 8). The psychophysical method measures perceptual responses of subject to the visual stimuli that are carefully controlled and measured by optical density (44a).

CONCLUSION

The identification of enzymes that catalyze the breakdown of carotenoids at a molecular level opens a new era in vitamin A research. In addition, the rapid progress in the methodology to assess the bioavailability of carotenoids, specifically stable-isotope dilution methods, allows us to understand the true vitamin A activity of dietary provitamin A carotenoids. Continued efforts to determine the functional bioavailability of carotenoids (such as antioxidant capacity, macular pigment density, etc.) may further improve our understanding of the bioavailability of carotenoids. However, we can now only grasp fragmented pieces of the numerous complex events influencing the absorption, metabolism, and transport of

carotenoids. Additional research is required to accurately predict the true bioavailability and biological functions of carotenoids and their metabolites in vivo.

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