

Beta-Carotene: The Controversy Continues

Lyn Patrick, ND

Abstract

The three β -carotene intervention trials: the Beta-Carotene and Retinol Efficacy Trial (CARET), Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), and Physician's Health Study (PHS) have all pointed to a lack of effect of synthetic β -carotene in decreasing cardiovascular disease or cancer risk in well-nourished populations. The potential contribution of β -carotene supplementation to increased risk of lung cancer in smokers has been raised as a significant concern. The safety of synthetic β -carotene supplements and the role of isomeric forms of β -carotene (synthetic all-trans versus "natural" cis-trans isomeric mixtures), in addition to the importance of the protective role of other carotenoids like lycopene and lutein, have become topics of debate in the scientific and medical communities. This review addresses the biochemistry and physiology of the cis versus trans isomers of β -carotene as well as relevant studies comparing the absorption and storage of the synthetic versus natural forms of β -carotene. In addition, the risk of potential pro-oxidant effects of synthetic β -carotene supplementation in intervention trials is evaluated.

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Introduction

More than 600 carotenoids have been isolated in nature, β -carotene being the best known.¹ Approximately 50 carotenoids have been found in the human diet and 20 have been identified in plasma and tissues.²

β -carotene has been shown to act as an immune modulator, quench singlet oxygen, and reduce peroxy radicals at a low partial oxygen pressure.³ β -carotene also enhances gap-junction communication⁴ and, in the rat model, induces hepatic enzymes that detoxify carcinogens.⁵ In both observational and case control studies, the intake of carotenoid-rich fruits and vegetables has been found to be inversely correlated with risk for cardiovascular disease.⁶ Numerous retrospective epidemiological studies have established an inverse relationship between dietary carotenoid levels and the incidence of specific cancers.^{7,8} Numerous animal and laboratory studies have substantiated β -carotene's ability to inhibit tumor cell growth and the progression of carcinogenesis.⁹

Lyn Patrick, ND – Associate Editor, *Alternative Medicine Review*; Private Practice, Tucson, AZ
Correspondence address: 540 W Prince, Ste A, Tucson, AZ 85705

Digestion and Absorption of Beta-Carotenes

Carotenoids are found in foods in a complex with proteins or in crystalline carotenoid complexes.¹⁰ Heating appears to improve the bioavailability of carotenoids by breaking down these complexes, as has been shown for lycopene in cooked versus raw tomatoes.¹¹ Typical dietary β -carotene intakes of American adults are 0.5-6.5 mg/day (833 IU-10,829 IU).¹²

Absorption of β -carotene and other carotenoids from vegetables is usually 5-30 percent of the absorption from synthetic supplements, due to the food matrix surrounding β -carotene. This matrix of fiber or protein must first be broken down by mastication, gastric acid, pancreatic enzymes, and bile acids.¹³

The Effect of Gastric pH

Gastric pH level has also been found to alter β -carotene absorption. In the Boston Nutritional Status Survey, conducted between 1981 and 1984, the median serum carotenoid values were lowest for those subjects with severe atrophic gastritis, a condition in which little or no hydrochloric acid is secreted by the stomach.¹⁴ Since 30 percent of the U.S. population over age 60 is estimated to have atrophic gastritis,¹⁵ the potential malabsorption of β -carotene in this population subset appears to be significant.

In another study of β -carotene absorption and gastric pH, the effect of omeprazole-induced hypochlorhydria on β -carotene absorption was investigated.¹⁶ The authors found that serum β -carotene concentrations were significantly lower at a higher gastric pH of 6.4 ± 0.3 (with omeprazole) than at a gastric pH of 1.3 ± 0.1 (without omeprazole). Lipid micelles containing carotenoids, formed in the duodenum as a result of fat digestion, release carotenoids into mucosal cells of the duodenum by passive diffusion, determined by the concentration gradients between the two.¹⁰ The

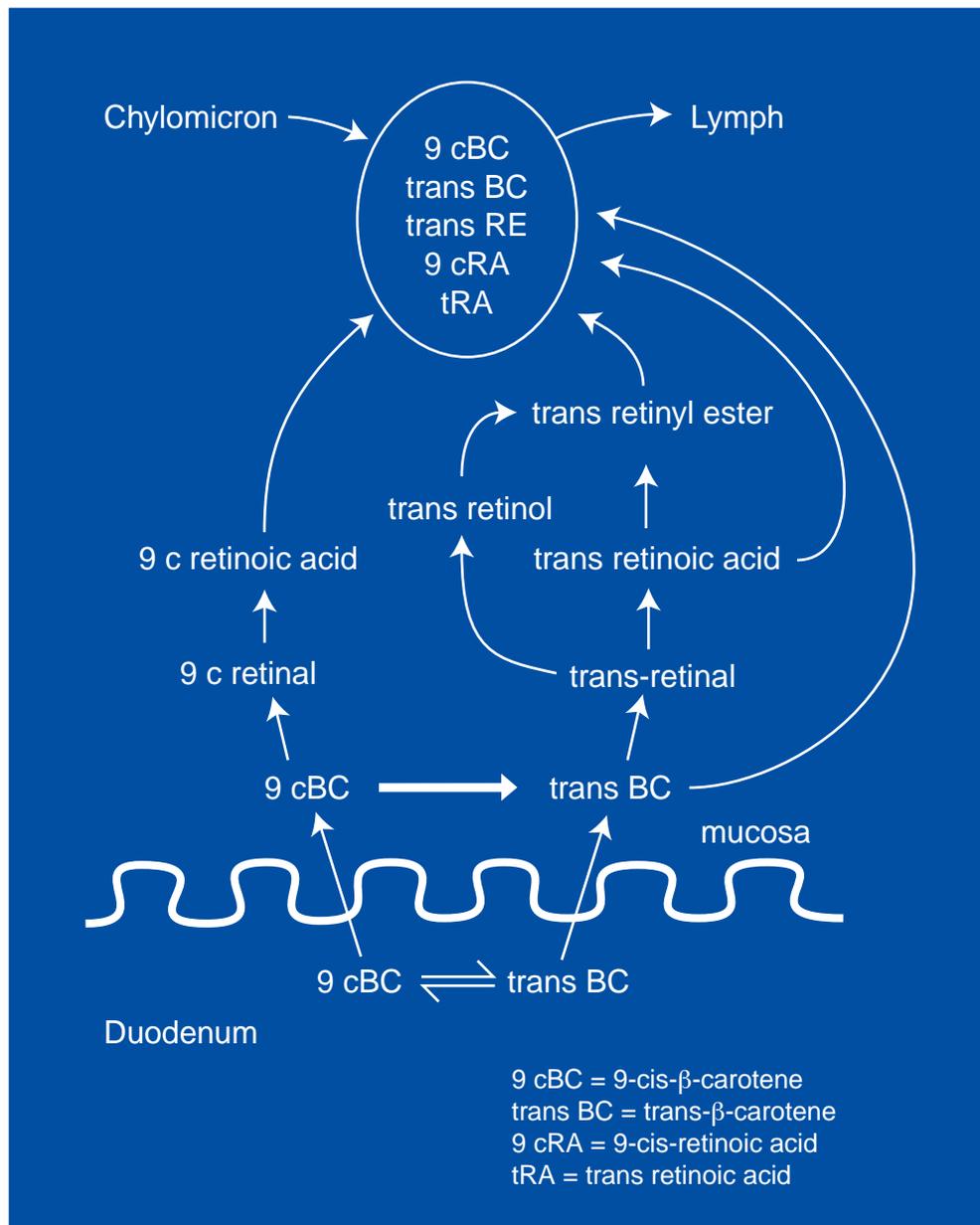
authors of the omeprazole study theorized that a higher gastric pH increased the negative surface charges of the carotenoid-containing micelle and the intestinal lumen, inhibiting passive diffusion.¹⁶ Saturation of these uptake mechanisms are estimated to occur only at intakes below 100 mg (166,000 IU),¹⁰ although absorption studies discussed later in this article appear to reveal significant problems with β -carotene absorption at dosage levels much lower than 100 mg.

The digestion of carotene complexes in the duodenum occurs as a result of the action of pancreatic lipase and bile salts,¹⁰ and absorption depends on the efficiency of lipid digestion; non-digested lipids interfere with carotenoid absorption.¹⁷ Similarly, bile acid sequestrant drugs, like cholestyramine, reduce carotenoid absorption as do other situations that interfere with micelle formation: malabsorption, intestinal parasites, and steatorrhea or fat malabsorption syndromes from pancreatic or gall bladder disorders.¹⁸ Certain forms of dietary fiber also appear to inhibit carotenoid absorption: when 12 grams of citrus pectin was added to a meal with 25 mg β -carotene, plasma β -carotene levels were significantly reduced.¹⁹

The Effect of Dietary Fat

Dietary fat is a factor in carotenoid absorption. High-fat diets (18-24 g fat with breakfast, 45 g fat with midday meal) produced better β -carotene absorption than low fat diets (no fat for breakfast and 6 g for midday meal) in test meals when subjects were given 45 mg β -carotene for five days.²⁰ Although the difference in absorption between the two groups was significant, the low-fat diet group still had a measurable and sustained rise in β -carotene levels, apparent after 13-15 days of treatment. Levels in the low-fat diet group, however, dropped back to baseline more quickly after treatment was discontinued (7-23 days vs 21-41 days post-discontinuation). Adding 18 g olive oil to a low-fat diet (7% of total calories)

Figure 1: Proposed Absorption Mechanism for Beta-carotene and Conversion to Vitamin A in Intestinal Mucosa.



Intestinal Absorption of Beta-carotene

In the small intestinal enterocyte, β-carotene can be transformed into vitamin A mainly as retinyl ester (20-75%) through cleavage of the β-carotene molecule. The majority of conversion to vitamin A takes place, not in the liver, but in the intestinal mucosa (Figure 1).²³ This cleavage depends on the vitamin A content of the meal and the vitamin A status of the individual.²⁴ In vitamin A-depleted subjects, synthetic β-carotene had 50 percent of the potency of retinol; in other words, 2 mg β-carotene was equivalent to 1 mg vitamin A.²⁵ However, β-carotene has not been shown to precipitate vitamin A toxicity, and it has been demonstrated in several species that, when dietary β-carotene increases, the regulatory mechanisms limit vitamin A production from carotenoids.¹⁸ β-carotene not converted to vitamin A is absorbed by the lymphatics, having been incorporated into chylomicrons as intact β-carotene or other non-vitamin A products of β-carotene cleavage.²⁶ The entire family of

improved carotene absorption from 5 to 25 percent in young African males.²¹ Absorption studies with low-fat diets have shown a minimum of 3-5 g per meal is necessary for any carotenoid absorption to occur.²²

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carotenoids is found in the bloodstream, incorporated into either chylomicrons or lipoproteins: very low density lipoproteins (VLDL), low density lipoproteins (LDL), or high density lipoproteins (HDL) – the majority being transported in LDLs.²⁷ Actual absorption levels of β -carotene have dramatic individual and inter-individual variation with reports in the literature varying from 10-90 percent.²⁸ Three- to four-fold variations in plasma β -carotene levels among individuals have been seen after a single dose of 30 mg (50,000 IU).²⁹ These results have been confirmed in studies that control for fat malabsorption, smoking, alcoholism, gastrointestinal disorders, and fat content of the test meals.²⁶ Absorption appears to be linear up to doses of 20-30 mg (33,000-50,000 IU) and then begins to become limited.¹⁰

Absorption studies exploring individual isomers are difficult given that some β -carotene is converted to vitamin A in the intestinal lumen and some is unabsorbed and lost in the feces as intestinal enterocytes are sloughed off.¹⁰ β -carotene isomers also appear to have a biphasic appearance in the plasma, possibly as a result of re-secretion of VLDL from the liver or storage in the intestinal epithelium; levels have remained high for three days after an oral dose of 120 mg (200,000 IU) or have risen 12 hours after a single dose (of 120 mg) and then again three or four days later.²⁶ The body also appears to have a mechanism for maintaining significant β -carotene reserves. When healthy males were fed low β -carotene diets they maintained normal blood β -carotene levels for 17 weeks.³⁰

Mechanisms of absorption in specific tissues are not well understood. High levels of carotenoids are found in the liver, adrenals, kidneys, ovaries, adipose tissue, and the macula of the eye.³¹ Significant correlations have been found between serum and breast tissue levels of retinoids, tocopherol, and eight carotenoids including β -carotene.³² Significant amounts of carotenoids have also been found

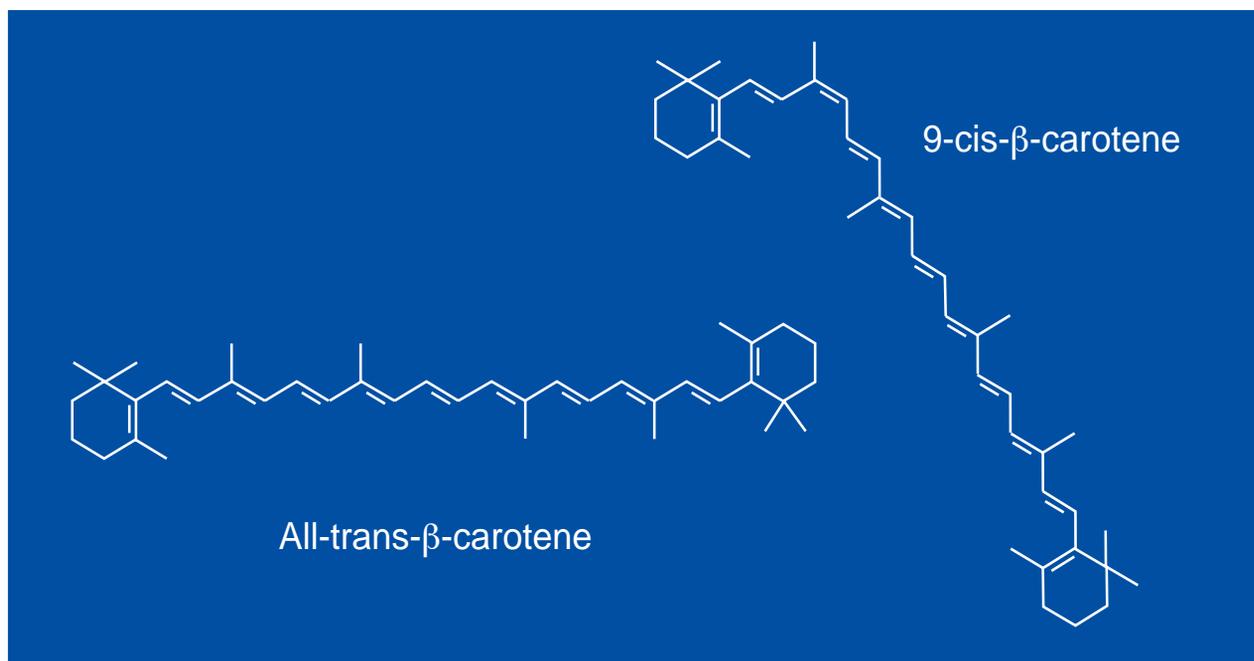
in the pineal gland and the corpus luteum of cattle.¹⁰ The accumulation in the adrenals, liver, testes, and ovaries may follow uptake of lipoproteins (LDL/HDL) but accumulation in other tissues, like the macula of the eye, appears to follow selective tissue uptake pathways which are still unknown.³³

Competitive Inhibition Among Carotenoids for Absorption

One difference between synthetic β -carotene and natural-source β -carotene involves the inclusion of other naturally-occurring carotenoids: lutein, canthaxanthin, lycopene, α -carotene, zeaxanthin, and cryptoxanthin in *Dunaliella* species, the algal form of commercially available mixed carotenoids.³⁴ These carotenoids have immune modulating activities of their own and the possibility that feeding high doses of purified β -carotene may inhibit absorption of other carotenoids has been investigated.

The studies examining competitive inhibition among carotenoids, however, have shown conflicting results. In a long-term study, the ATBC trial, commonly known as the Finnish Smokers Study, a 6.7-year supplementation with 20 mg (33,000 IU) synthetic all-trans β -carotene resulted in a significant decrease (\cong 11%) in serum lutein levels.³⁵ Other studies have not shown evidence of competitive inhibition. The Physicians' Health Study, a 12-year treatment with 50 mg (83,000 IU) all-trans β -carotene dosed every other day did not show changes in any other serum or plasma carotenoid levels.³⁶ Two polyp-prevention trials, one with 25 mg (42,000 IU) synthetic β -carotene for four years³⁷ and the other supplementing 20 mg (33,000 IU) synthetic β -carotene for two years³⁸ found different results: the four-year study showed no effect of β -carotene supplementation on other carotenoid levels while the two-year study found increases in serum lycopene and α -carotene levels as a result of β -carotene supplementation. Another

Figure 2: Cis and Trans Isomers of Beta-Carotene.



long-term β -carotene study, employing doses of 50 mg (83,000 IU) for five years, found no significant effect on plasma carotenoid levels, with the expected exception of the α - and β -carotene found in the supplement.³⁹

The only negative carotenoid interactions have been found in short-term studies where varying levels of lutein and β -carotene have been fed simultaneously. When lutein was the predominant carotenoid, β -carotene absorption from a 22.5 mg (37,000 IU) β -carotene meal was inhibited.⁴⁰ β -carotene also appears to inhibit lutein absorption, although at insignificant levels, in intervention³⁵ and in single-feeding⁴¹ trials. Although these negative interactions have not been borne out in the majority of long-term supplementation studies with β -carotene, they are important to investigate due to the role lutein plays in macular function and the provitamin activity of β -carotene in retinoid metabolism.⁴⁰

Isomers of Beta-carotene: 9-cis Versus All-trans and Differences in Absorption

Isomers of β -carotene are found in varying forms in nature; each double-bond in the carbon chain of a carotenoid can exist in either the trans or the cis configuration, the cis configuration being less stable than the trans (Figure 2). Cis isomers are more polar, less crystallized, and more soluble in oil than the trans isomers.^{42,43} The all-trans, 9-cis, 13-cis, and 15-cis isomers have been identified in both food sources and human plasma in varying amounts depending on the source. Raw carrots, tomatoes, and sweet potatoes appear to be devoid of the cis isomer;⁴⁴ 98 percent of the β -carotene in raw carrots is in the trans-form.⁴⁵ The 9-cis-carotenoid isomers in foods appear to increase with processing and heating but multiple studies indicate the 9-cis-carotenoids are not absorbed as easily or are absorbed through different mechanisms than the all-trans isomers.¹³

The majority of carotenoids in nature occur in the all-trans form, which is

molecularly identical to synthetically produced all-trans- β -carotene.¹ All-trans β -carotene can isomerize under exposure to heat and with oxidation¹ and multiple studies addressed in this article document the isomerization of the cis- β -carotenes (in algal β -carotene) to the trans form in the human intestine.

The cis-isomers have been reported to account for less than five percent of human plasma β -carotene but a larger proportion (10-25%) of the total carotenoid content of human tissue; 9-cis- β -carotene makes up 25 percent of the total β -carotene content of the liver and 10 percent in the adrenals. Cis-isomers were also found in significant amounts in the kidneys, testes, ovaries, and fat tissue.³¹ The trans-isomer is the most common form in human tissue, comprising up to 60 percent of the total β -carotene content.³¹ However, significantly higher levels of the 9-cis isomer were found in the breast adipose of women with a history of benign breast lesions when compared to women with a history of breast cancer.³²

The debate over the differences between synthetic and food-source (or naturally-derived commercial – from the algal *Dunaliella* species) β -carotene centers around the role of the cis-isomers, which are absent from synthetic all-trans β -carotene. The importance of the 9-cis isomer has been linked to the fact that it is a direct precursor in the intestinal enterocyte to 9-cis retinoic acid.⁴⁶ Retinoic acid acts as a hormone in signaling processes where it binds to nuclear receptors and controls normal reproduction and maintenance of epithelial tissue.⁴⁷ Retinoids are also involved in preventing carcinogenesis, inhibiting squamous metaplasia, acting as a chemopreventive agent in epithelial carcinogenesis, and as a differentiating agent in acute promyelocytic leukemia.⁴⁷ Specifically, 9-cis retinoic acid binds to human nuclear retinoic acid receptor (RAR) and retinoid X receptor (RXR- α) and plays a significant role in the expression of normal epithelial and squamous tissue growth. The 9-cis retinoic acid isomer

has been studied as an antitumor agent in combination studies with either tamoxifen⁴⁸ or raloxifene⁴⁹ in experimental mammary carcinogenesis; both studies found significant inhibition of mammary tumor growth when compared to the anti-estrogens alone. Significant inhibitory effects on head and neck squamous cell carcinoma cell lines have also been found with 9-cis retinoic acid.⁵⁰

9-cis retinoic acid is formed only from 9-cis β -carotene, while all-trans β -carotene has been shown to be transformed into only all-trans retinoic acid.⁵¹ The 9-cis isomer of β -carotene appears to be isomerized in the intestinal mucosa to the all-trans isomer⁵² and theoretically, all-trans retinoic acid can be metabolized into 9-cis retinoic acid. Although this has been demonstrated both *in vivo* and *in vitro*, this pathway is not well understood and has not been demonstrated in the human gut.⁴⁶

In vitro, algal extracts of *Dunaliella* containing relatively equal mixtures of cis and trans isomers had a greater ability to prevent methyl-linoleate peroxidation than the synthetic all-trans β -carotene.⁵³ The ability of the trans and cis isomers of β -carotene to prevent lipid peroxidation has also been studied in an animal model.⁵⁴ Rats were fed 1g/kg β -carotene either in a *Dunaliella* extract of 75-percent 9-cis β -carotene or a synthetic all-trans isomer, and both groups were fed oxidized soy oil. Both carotenoid isomers prevented lipid peroxidation to the same extent; however, the trans isomer resulted in a significant depletion of hepatic carotene stores while the 9-cis isomer conserved liver carotene stores comparable to the levels in rats fed fresh soy oil.

As a result of the conflicting data between the cancer-protective effect of dietary carotenoid consumption and the subsequent negative results of supplementation trials using all-trans isomers of β -carotene, interest in the absorption and biochemistry of the β -carotene isomers has increased. Research has centered on the absorption and utilization of the three main isomers found in natural sources:

Table 1: Studies of Beta-Carotene Absorption.

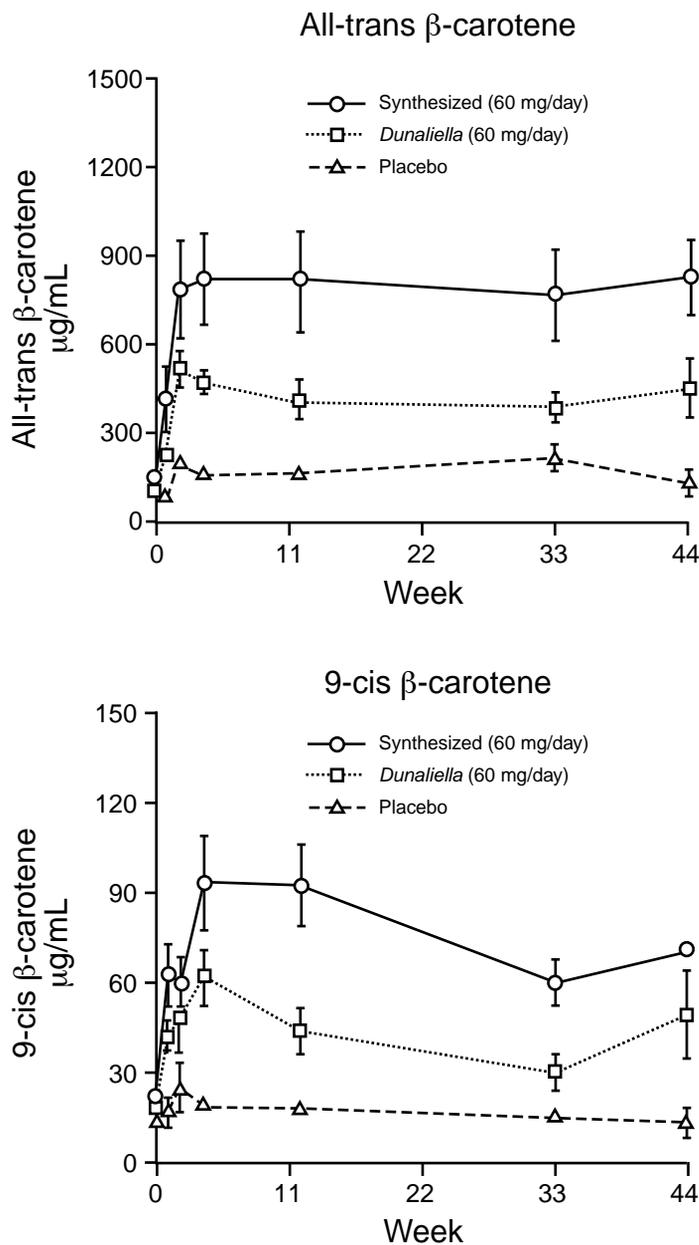
Study	Data	β -carotene absorption
Gaziano, et al. ⁵⁶	24 adult M + F for 6 days 100 mg synthetic vs. 66 or 100 mg algal source (trans/cis ratio=50:50)	160% increase in plasma β -carotene with synthetic, all trans form. 87% increase in plasma β -carotene with natural algal form
Jensen, et al. ⁴⁵	16 adult M and F for 7 days 24 mg β -carotene algal source: (trans/cis ratio=40:60) 24 mg β -carotene raw carrot: (98% all-trans)	algal source: serum cis-carotenoid increase 9.6% of total serum β -carotene carrot source: serum cis- carotenoid: 7.1% total serum β -carotene
Johnson, et al. ⁵⁷	15 M single dose of 120 mg β -carotene containing either all-trans β C or 80% 9-cis β C/20% all-trans	all-trans β C well absorbed in 7 of 15 no increase in all-trans β C with mixture
You, et al. ⁵²	3 adult M and F single dose 1 mg β -carotene (99.4% cis)- radioactively labeled (¹³ C)	>95% of 13 C β -carotene in plasma found as trans isomer (14-52%) and retinol
Tamai, et al. ⁵⁸	30 adult M 60 mg of either: algal β -carotene (trans/9-cis ratio=50:50) vs. all trans or placebo – 44 weeks	Levels of both cis (77%) and trans (116%) isomers significantly higher with all-trans β -carotene

all trans, 9-cis, and 13-cis β -carotene.

Data from absorption studies show clear differences in absorption among the isomeric forms. Multiple studies have compared human absorption of synthetic all-trans β -carotene with a natural isomeric mix of all-trans and 9-cis β -carotene from algal sources (*Dunaliella* species). The ratios of all-trans to 9-cis carotenoids in the different species of *Dunaliella* vary from 40:60 to 54:37.¹³ Consistently in the studies evaluated in Table 1, the levels of serum and plasma all-trans and 9-cis β -carotenoids were greater after feeding either synthetic all-trans β -carotene or, in the study by Jensen,⁴⁵ raw carrots containing at least 98-percent all-trans β -carotene compared to an algal extract.

Jensen gave either 207 g raw carrot (24 mg β -carotene) containing 98-percent all-trans β -carotene or an algal-source β -carotene (24 mg from *Dunaliella*) to 16 healthy adults and serum levels of all-trans and cis β -carotene were compared after seven days. The absorption of β -carotene from the raw carrot was greater than from the algae and the rise in serum cis β -carotene was significantly greater in those fed carrots than those who received algal carotene capsules. Despite the 50:50 trans-to-cis ratio of the algal carotenes, the absorption rate of cis-carotenes in the serum was only 9.6 percent of the rise in total serum β -carotene level, only slightly more than the rise in the serum level of cis β -carotene (7.1%) with raw carrot. There was a constant serum trans:cis ratio of 11:1 after *Dunaliella*-derived β -carotene ingestion indicating what other

Figure 3: Changes in Plasma Levels of Beta-carotene Isomers with Synthetic vs Naturally-derived Beta-carotene.



From: Tamai H, Morinobu T, Murata T, et al. *Lipids* 1995; 30: 493-498. copyright ©, 1995 *Lipids*. All rights reserved.

to cis isomers has been shown to be between 10:1 and 50:1 after consumption of 5.6 mmol/kg natural β -carotene consisting of 54-percent all-trans β -carotene and 37-percent 9-cis β -carotene.⁴⁶

A study by Gaziano et al⁵⁶ examining the difference in absorption between all-trans β -carotene and algal β -carotene from *Dunaliella salina* (50:50 trans:cis ratio) found synthetic β -carotene produced a higher plasma level of all-trans β -carotene and a five-fold increase in 9-cis β -carotene, while the natural algal form produced only a 3.7-fold increase in 9-cis isomer. As a result of the significant increase in levels of the plasma trans-isomers in the synthetic β -carotene group, however, the trans to cis isomer ratio was much higher than was normally seen: 15.1 compared to a baseline of 10.3. The natural source or algal β -carotene did not alter the plasma isomer ratios. After 23 additional days on an alternate daily dosing of 50 mg synthetic β -carotene or a daily dosing of either 66 mg or 100 mg algal-derived β -carotene, there were no real changes in the isomeric ratios or the total plasma β -carotene ratios of those given the algal source. Total β -carotene plasma levels in subjects given synthetic β -carotene were higher during the entire 30 days of the trial. It is also interesting to note there were no differences in the plasma levels of β -carotene achieved with either 66 mg (110,000 IU) or 100 mg (166,600 IU) of the natural β -carotene.

Johnson⁵⁷ studied the absorption of a "natural" algal carotene mixture containing 80-percent 9-cis β -carotene and equal amounts of all-trans β -carotene, α -carotene, and 13-cis β -carotene versus a mixture containing 93-percent all-trans β -carotene

researchers have found, a higher ratio of trans to cis isomers in the serum, plasma, and chylomicrons after feeding algal-source β -carotene.⁵⁵ In chylomicrons, the ratio of trans

and 7-percent α -carotene, 9-cis and 13-cis carotenes. The two mixtures were given to 15 male volunteers in single doses one week apart. Serum levels in the group given the 9-cis β -carotene mixture did not show appreciable changes in the all-trans isomer and the levels of 9-cis β -carotene were significantly raised at only one point during the 24 hours of measurements. The serum levels following the 93-percent all-trans mixture were significantly higher for the all-trans isomer and showed no increase in the 9-cis isomer.

Tamai⁵⁸ gave 38 male volunteers either 60 mg synthetic all-trans β -carotene or 60 mg algal β -carotene (trans-to-cis ratio 50:50) for 44 weeks. His group found the levels of all-trans β -carotene in the plasma of those who had taken the synthetic form were twice as high as for those who had taken the natural form. Surprisingly, he also found the synthetic β -carotene group had a significantly higher level of plasma 9-cis β -carotene, even though there were no 9-cis β -carotene isomers in the synthetic form (100% all-trans β -carotene) (Figure 3). The authors concluded that isomerization from the all-trans to the 9-cis form probably takes place in the body during or after absorption and that the 9-cis form accumulates in tissue as a result.

A study was published one year after Tamai's work that appears to solve the all-trans and 9-cis isomerization question. A group at Cornell University⁵² gave three healthy adults a 1 mg (1666 IU) single dose of 99-percent cis β -carotene labeled with a radioactive tracer (¹³C) and found over 95 percent of the cis isomers had been isomerized to trans β -carotene or transformed into retinol prior to entering the bloodstream. Using calculations the authors admit "may be underestimates" they speculated that 14-52 percent of the cis β -carotene had been isomerized to the all-trans form.

They also compared the absorption of the labeled cis β -carotene by giving a single dose of unlabeled cis β -carotene eight months later.⁵² As a result of the comparison data, they

produced three provocative points. First, this was the first definitive proof that cis β -carotene is isomerized in the intestinal lining to the trans isomer before absorption. Second, the authors suggest that greater amounts of all-trans β -carotene are absorbed and secreted into chylomicrons after cis β -carotene dosing than occurred after an equivalent dose of all-trans β -carotene, alluding to the fact that (unlike other researchers' findings) the cis isomer may actually be better absorbed. Third, most of the radioactively-labeled retinol produced by the study subjects was made from the labeled trans-isomer rather than being produced directly from the labeled cis-isomeric form of β -carotene, pointing out a more efficient mechanism for retinol production by transforms of β -carotene. They concluded the isomerization process could serve as a control mechanism for the production of 9-cis retinol. Because this study used a very small dose of β -carotene (1666 IU), absorption mechanisms may not be reproducible with higher doses.

The results of this study point out the gaps in our knowledge about cis β -carotene and its relationship to β -carotenoid absorption and metabolism. They also provide an explanation for the previous dosing studies in which 9-cis β -carotene blood levels were always lower than dosing levels, a result of the isomerization of the cis isomer to the trans isomer in the intestine.

Although 9-cis β -carotene (along with the 13-cis and 15-cis isomers found in food and naturally-occurring substances) may serve an important function in human physiology that cannot be replaced by synthetic β -carotene, the consequences of using all-trans synthetic β -carotenes are at this point unknown. There is evidence that 13-cis β -carotene can be produced from all-trans β -carotene in significant quantities: as much as 10 percent of total β -carotene intake.⁵⁹ Certainly, using the β -carotene carotenoid exclusively may be problematic as there appears to be a possible competitive inhibition between β -carotene and

lutein. However, as discussed previously, long-term β -carotene studies have not found such competition. Whether or not the lower lutein levels seen in the Finnish Smokers Study are relevant is unknown.¹

Beta-Carotene Trials

Intervention studies with β -carotene have not shown the efficacy predicted by observational and animal studies. The well-publicized intervention trials with β -carotene, the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC)⁶⁰ and the Beta-Carotene Retinol Efficacy Trial (CARET),⁶¹ were designed to assess the effects of tocopherol, β -carotene, and retinol in populations at high risk for lung cancer – former and present smokers and asbestos-exposed workers.

The ATBC trial (Finnish Smokers Study) looked at the effect of supplementation of alpha-tocopherol (50 mg) and β -carotene (20 mg), alone or in combination, in 29,133 smokers for 5-8 years. At the conclusion of the study, those receiving β -carotene, alone or in combination with tocopherol, had a 16-percent increased incidence of lung cancer compared to those receiving tocopherol alone or placebo. The elevation of risk was restricted only to those who were smoking at least one pack of cigarettes daily; those smoking less than one pack daily had no increase in lung cancer risk and β -carotene had no effect on other types of cancers (prostate, bladder, stomach, colorectal).

The CARET study evaluated the effect of 30 mg β -carotene and 25,000 IU retinyl palmitate versus placebo in 18,314 men and women.⁶¹ Twenty-two percent of the study population were occupationally asbestos-exposed, 39 percent were former smokers, and 60 percent were current smokers. The trial was ended two years early, after approximately four years, when the incidence of lung cancer in the intervention group was found to be 28-percent

higher and the incidence of mortality 17-percent higher than the placebo group. Those who were smoking during the study had an even higher risk of lung cancer: a relative risk of 1.42 (42-percent increase in incidence). In the participants who had stopped smoking at least two years prior to entry, the combination of β -carotene and retinol had a protective effect that did not reach statistical significance – a relative risk of 0.80 for diagnosis of lung cancer during the study period.

A third study, the Physicians' Health Study (PHS), assessed the effect of 50 mg β -carotene every other day and 325 mg aspirin alone or in combination for 12 years against a placebo group in a population of 22,071 physicians.⁶² The endpoints in this study were cancer and cardiovascular disease incidence and mortality. Although the population of current smokers in this study was smaller (11%), the population of former smokers (39%) was substantial. There was no increased incidence of

Table 2: Treatment Groups in Linxian Study.⁶³

Treatment Group	Treatment (daily dosage)
1	retinol 5,000 IU zinc 22.5 mg
2	riboflavin 3.2 mg niacin 40 mg
3	ascorbic acid 120 mg molybdenum 30 mcg
4	β -carotene 15 mg selenium 50 mcg α tocopherol 30 mg
Treatment group combinations:	1+2, 1+3, 1+4, 2+3, 2+4, 3+4, 1+2+3+4, placebo

Table 3: Comparisons of Intervention Trials with Beta-Carotene and Other Antioxidants.

Study	PHS ⁶²	ATBC ⁶⁰	CARET ⁶¹	Linxian ⁶³
treatment	*Aspirin 325 mg *β-carotene 50 mg q other day *combination *placebo	*α-tocopherol (50 mg) *β-carotene (20 mg) *combination *placebo	*retinol (25,000 IU) plus β-carotene (30 mg) *placebo	*β-carotene (15 mg) *selenium (50 mcg) *α tocopherol (30 mg)
% former plus current smokers at initiation	50%	100%	80%	30%
Plasma β-carotene levels after intervention (micrograms/ml)	1.2	3.0	2.1	0.86

mortality or cancer diagnosis in the β-carotene population, and only those in the lowest quartile for baseline plasma β-carotene experienced a protective effect of β-carotene for prostate cancer.

A fourth study, from Linxian, China, compared the cancer-protective effects of four supplemental vitamin and mineral combinations.⁶³ The study included 29,584 adults divided into eight groups: either placebo or combinations of retinol, zinc, riboflavin, niacin, ascorbic acid, molybdenum, β-carotene, selenium, and α-tocopherol, in a complex 2 x 2 x 2 x 2 factorial design that included all possible combinations of the four groups in paired sequences (Table 2).

The study was conducted for five years. At the conclusion the group taking 50 mcg selenium, 30 mg α-tocopherol, and 15 mg β-carotene, when compared to the other intervention groups and placebo, had a reduction in cancer risk of 13 percent, primarily due to a decrease in the incidence of gastric cancer.

Comparisons of these trials are useful even though they involved different treatment protocols, patient populations, and endpoints (Table 3).

The Linxian study used the smallest dosage of β-carotene (15 mg) and resulted in the lowest mean level of plasma β-carotene (0.86 mcg/mL). The incidence of lung cancer was reduced by 55 percent although, because there were only 31 total deaths from lung cancer in the study, the statistical power of lung cancer incidence was low.⁶³ The number of patients in this trial who were “ever smokers” (current or former smokers) was 30 percent, compared to 50 percent in the PHS trial, 100 percent in the ATBC trial, and 80 percent in the CARET trial.⁶⁰⁻⁶³

The ATBC study, providing 20 mg β-carotene/day, resulted in the highest mean plasma levels of 3.0 mcg/mL, a level 18-times higher than the mean baseline. Higher plasma levels of β-carotene in this study, however, had no relationship, positive or negative, to lung cancer incidence.⁶⁰ In all three intervention studies where the incidence of smoking was highest (ATBC, PHS, CARET), the overall mortality and the incidence of cancer were inversely correlated with the baseline levels of β-carotene, independent of treatment protocol.¹ The implications of the ATBC and CARET studies – that β-carotene acts as a co-carcinogen – have been criticized because the

smokers in the studies had been smoking for many years (36-pack-year history was the average in the ATBC study) prior to study entry. The critical stages of initiation and promotion of tumors, stages that antioxidants have been shown to inhibit,⁶⁴ may have already been bypassed at the initiation of the trials.¹

Carotenoids as Pro-oxidants

Carotenoids, as a class, are particularly vulnerable to free radical attack due to their long chains of conjugated double bonds.⁶⁵ A pro-oxidant effect has been seen in *in vitro* and animal studies, both in high concentrations and in the presence of tocopherol deficiencies.⁶⁶ The gas phase of cigarette smoke contains high levels of oxidants (nitric oxide) that have been shown to interact with lipid membranes to induce lipid peroxidation and protein oxidation to form protein-bound carbonyl groups.⁶⁷ The protein-carbonyl groups result in the inactivation of crucial enzymes such as creatine kinase and lecithin-cholesterol acyltransferase.⁶⁷

Carotenoids interact with cigarette smoke-containing oxidants. Depletion of carotenoids and tocopherol, particularly trans- β -carotene, alpha-tocopherol, and lycopene, has been demonstrated in cigarette smoke-exposed human plasma.⁶⁸ As β -carotene acts as a scavenger of nitrogen oxides in cigarette smoke, β -apo-carotenals and other carotene oxidation products are created that, if not effectively neutralized by other antioxidants (specifically tocopherol and ascorbate), may initiate cell damage that could lead to neoplasm.⁶⁵ Multiple studies have substantiated that tocopherols (both alpha and gamma) protect carotenoids from auto-oxidation.^{69,70} Ascorbate also acts to protect both tocopherol and β -carotene from oxidative damage and has been shown to preserve β -carotene in oxidized human LDL.^{71,72}

The theory that β -carotene becomes an oxidant in the plasma and possibly tissues of smokers appears to be probable given the low antioxidant levels of smokers' blood.⁶⁸

Whether or not these carotenoid radicals can initiate cancer is, however, disputed. Baker⁷³ and colleagues examined a model of liposomes exposed to cigarette smoke. They found that β -carotene neither enhanced lipid peroxidation in membranes nor contributed to the depletion of other antioxidants. They concluded, "The data strongly suggest that, although β -carotene is readily oxidized by smoke, pro-oxidant effects are unlikely to account for the apparent enhancement of lung cancer in smokers taking this supplement."

One actual benefit of β -carotene's pro-oxidant effect may be its action in tumor cells. Carotenoids as a class appear to act as oxidizing agents selectively in tumor cells by increasing the production of heat shock proteins which ultimately enhance tumor cytotoxicity.⁷⁴

Conclusion

The issues of absorption and metabolism of carotenoids are full of unanswered questions. The significant inter-individual and intra-individual variations in absorption of β -carotene indicate the possible effect of medications, individual variations in gut pH, sub-clinical fat malabsorption, and intestinal dysbiosis that exist outside of overt fat malabsorption and gastrointestinal disease.

Competitive inhibition of different carotenoids appears to occur in single-dosing studies but only bears out in long-term human studies that involve smoking populations. It is not clear whether there is an interaction between synthetic β -carotene and cigarette smoke that may have caused lower lutein levels in the Finnish Smokers Study.

The efficient uptake of synthetic all-trans β -carotene and isomerization in the gut to 9-cis β -carotene appears to make the synthetic form more desirable for effective absorption. But the tendency of synthetic β -carotene to alter normal serum trans/cis ratios in favor of the trans isomer may not be a beneficial effect. If, as theorized by You et al,

the preferred absorption of all-trans isomers is a mechanism to control the production of 9-cis retinoic acid by the 9-cis β -carotenoid isomer, then artificial down-regulation of this important chemopreventive growth-regulator may not be wise.

Clearly, more research is needed to fully understand the antioxidant mechanisms in β -carotenoid physiology. The isomerization of all-trans β -carotene in the gut lumen to 9-cis β -carotene is not a thermodynamically favored reaction and may be catalyzed enzymatically.⁷⁵ The 9-cis β -carotenoids do appear to have an advantage as antioxidants in preventing depletion of hepatic β -carotene stores in the rat model; however, whether or not cis β -carotenoids act as antioxidants at the human gut surface is unclear.

The transport mechanisms of 9-cis β -carotenoids may be different than those defined for all-trans β -carotenoids and may explain their appearance in tissues at greater levels than those found in plasma. Again, if all-trans β -carotene is re-isomerized in the tissues to the 9-cis form, unknown enzymes may catalyze these reactions and we have little if any information about them.

The possibility that β -carotene acts as a pro-oxidant and co-carcinogen in the lung tissue of smokers who smoke more than one pack per day has not been ruled out and the use of β -carotene (natural or synthetic) in heavy smokers or those exposed to significant amounts of airborne nitric oxides must be questioned, as we do not have enough information about the antioxidant deficiencies inherent in this population.

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