Carotenoids: More than just beta-carotene

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Fruits and vegetables of the human diet contain many of the over 600 carotenoid pigments that have been identified in plants. Led by work with beta-carotene, researchers have constantly been learning more about the metabolism of these compounds in the human body. Research work is now expanding beyond beta-carotene in an effort to understand what happens to all the pigments found in the human diet. This discussion briefly looks at research results on the carotenoids found in human serum as well as the effects of supplementation. Recent confusing results from large intervention trials with beta-carotene and lung cancer incidence are emphasized in relation to supplementation doses and beta-carotene source (synthetic vs. natural). The summation of results emphasizes the importance of the broad spectrum of carotenoids in the diet and relates to supplementation products currently being designed for the marketplace.

Key words: carotenoids, beta-carotene, alpha-carotene, lutein, lycopene, disease, cardiovascular, cancer.

Introduction

The news media are reporting more and more frequently on scientific studies which demonstrate the effects of various nutrients on human metabolism and health. The diet recommendations made in these reports can vary widely and change rapidly. A prime example of this can be seen in the story of beta-carotene, which has gone from being used as a colorant, to a valuable provitamin A, to a promising antioxidant, to a major disease preventative, to being implicated in causing disease, all in the space of some 20 or so years. However, this attention to a single molecule has limited the understanding of the overall interaction of carotenoids in human metabolism. This report will outline what the carotenoids are, and how they act in human nutrition, with an emphasis on the relationship of beta-carotene to disease, which has received so much press coverage. The results will be considered in relation to the design of carotenoid formulations for dietary supplements.

Over 600 varieties of carotenoid pigments have been described and that number is likely to increase.[1] In nature they are responsible for many of the yellow to orange to red colours common to many organisms. They are produced by plants, bacteria and some fungi. Though animals are not capable of carotenoid synthesis, they are often taken up from the diet and used to colour body parts; for example, feathers in birds and skin and flesh in fish.[2]

It has been estimated that the annual production of carotenoids on the planet is roughly 100 million tons per year.[2] In plants they have been shown to serve a number of different functions.[3] Primarily they act as antennae pigments for the process of photosynthesis. Some carotenoids are integral parts of the photosystems and absorb light energy that is transferred to chlorophyll to power the photochemistry. Another important function is what is termed the 'xanthophyll cycle', where three different carotenoids (violaxanthin, antheraxanthin and zeaxanthin) act through interconversion to dissipate excess light energy that would in other circumstances damage the photosystems.[4] It is not yet known if this same process is acting in areas where these xanthophyll pigments are concentrated in humans; for example, the retina of the eye. It remains an intriguing hypothesis.

All of these activities in plants are due in large part to the basic structure of carotenoid molecules. Each carotenoid is a variation on a basic 40 carbon skeleton formed from isoprenoid subunits. This skeleton can be modified with oxygen containing groups (the xanthophylls), with different cyclization at the ends and with different levels of hydrogenation to yield the wide variety of carotenoids observed.[1] The most characteristic feature common to the carotenoids is the long system of conjugated double bonds. This is what allows the energy absorption and transfer that is the basis of their chemical reactivity and action in biological systems, not to mention the beautiful colours we see in nature. The structures of a few of the common carotenoids are given in Fig. 1

In addition to the basic chemical formula, there is another level of complexity of structure that has been shown to have an effect on the activity of carotenoids. This is the isomerization around the double bonds which can change the shape of the molecules. Figure 1 gives an example of this in the comparison of the *trans* and *9-cis* forms of the beta-carotene molecule. (In current chemical usage these conformations are termed E and Z, respectively, but these designations are not often used in nutrition literature.) Theoretically, there are thousands of different isomeric forms that could exist, but most are unstable and only a few isomers of each carotenoid are commonly found in nature. The majority of synthetically produced carotenoids are of the all *trans* conformation and these have received most of the research attention.

The analysis of carotenoids takes advantage of their characteristic absorption of light. Spectrophotometric analysis is

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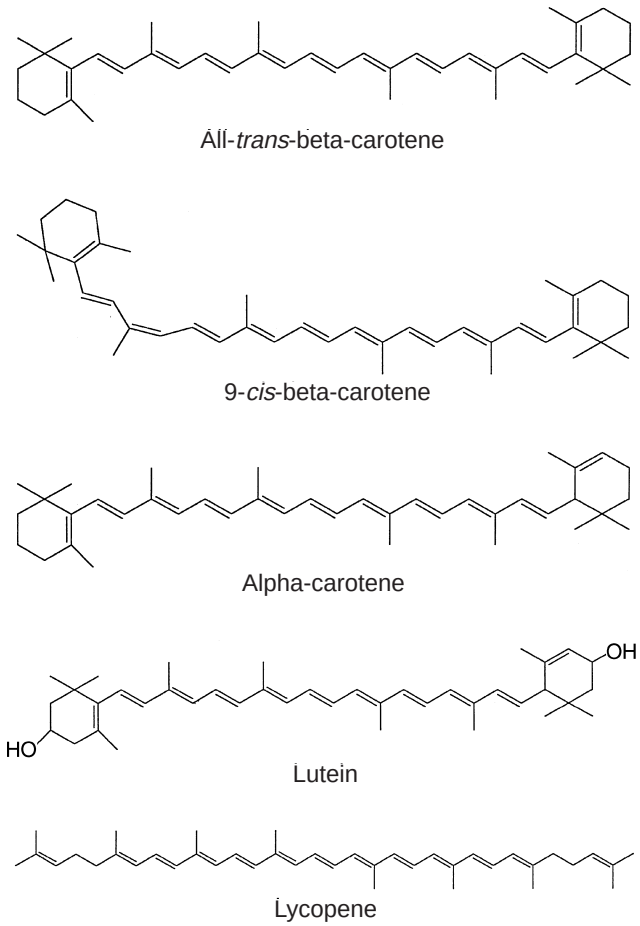

Figure 1. Structures of some common carotenoids.

able to quantify carotenoids extracted from food, serum or tissues. In recent years sophisticated separations using HPLC (high performance liquid chromatography) have allowed very precise determinations of carotenoid levels and revealed tremendous diversity in fruits and vegetables that make up human diets around the world. In 1993 a carotenoid food composition database was first published by the US Department of Agriculture and the National Cancer Institute and the data critically evaluated.[5] This database covered five of the most commonly occurring carotenoids: beta-carotene, alpha-carotene, lycopene, lutein+zeaxanthin and beta-cryptoxanthin. Before this time it was very difficult to be precise in determining the carotenoids that were actually consumed in various diets when plant sources varied widely. Comparative data from another laboratory that includes a wider range of carotenoids is being published by Ben-Amotz *et al.*[6] This data confirms extensive diversity in both the carotenoid quantity and type in different fruits and vegetables.

Carotenoids in nutrition

From early in the 1900s it was known that there was a fat soluble principle that was essential for life; it was termed vitamin A. A link between this vitamin and carotenoids was discovered by Von Euler in 1929 and with later work it was established that many of the carotenoids could be metabolized by the body to form vitamin A.[7] This pushed interest in this group of natural colorants forward and in the 1950s, industrial synthetic processes were created to commercially produce beta-carotene and make it available in a pure and relatively inexpensive form. These enterprises continued to push research and, therefore, until recent years the vast majority of effort and dollars in carotenoid research has gone into work relating to beta-carotene. The research bias served to greatly expand our knowledge in this one area but an understanding of the other carotenoids has lagged behind.

Khachik *et al.*[8] have worked extensively on elucidating the carotenoid content of human serum. Of some 50–60 different carotenoids that appear in food in quantities high enough to be measured, 34 have been detected in serum, including 13 geometrical isomers and eight metabolites. This work also examined human breastmilk where the same 34 carotenoids were identified, but in four- to 22-fold lower concentrations. The mechanisms of absorption, metabolism and transport of carotenoids are beyond the scope of this paper, but a review by Parker covers this area.[9] Briefly, once released from food, lipid droplets containing the carotenoid pigments form micelles which are absorbed in the duodenum into the intestinal mucosa. These are packaged into chylomicrons and transported in the lymph to the bloodstream. There is much yet to be understood of the complexity of these absorption and metabolic processes in human populations. Two examples that demonstrate this are matrix effects and gender differences.

In a study with a population of Indonesian women it was shown that there was a lack of improvement in vitamin A status in a group fed green leafy vegetables as compared with another fed a manufactured wafer containing a similar amount of beta-carotene in oil solution.[10] Forman *et al.* have shown in another study that there are cyclical fluctuations of plasma carotenoid concentrations in women that vary in conjunction with phase of the menstrual cycle.[11] This factor has seldom been taken into account in clinical studies. Finally, it appears that the different geometric isomers of a single carotenoid, for example the *trans* and *cis* forms of beta-carotene, can be metabolized very differently in the body, and these differences affect their distribution in tissues and their activity.[12]

Thus, it is firmly established that the carotenoids are taken up by the body; the question remains as to what they do once they get there. Again, there are a number of reviews that cover the areas of carotenoid metabolism, function and action, and to cover them all is beyond this paper.[9,13] However, two important actions which have received attention will be mentioned. First is the previously mentioned function of some carotenoids as a provitamin A. Not all carotenoids display this activity but two that do, alpha-carotene and beta-carotene, are among the most commonly occurring in food and serum. Most is known about beta-carotene which, through either a central or excentric cleavage of the molecule,[14] forms one or two molecules of retinal that are further converted to retinol or retinoic acid.[2] The USA Recommended Daily Allowances for vitamin A of 5000 IU is provided by only 3 mg of beta-carotene. A second major action of carotenoids is as an antioxidant. This has been shown in both *in vitro* and *in vivo* animal systems, most often through the inhibition of lipid peroxidation in the presence of carotenoids.[13] It is this second action which has sparked the most research interest due to the possible association with disease.

Carotenoids and disease

There have been a great many studies associating carotenoid intake in the diet to health effects in humans and to a wide variety of disease incidences (e.g. heart, cancer, eye, stroke and ageing), either through food records or actual blood or tissue measurements. These observational studies have been the basis for the recommendations from various nutrition and governmental agencies of five or more servings of fruits and vegetables a day for a healthy diet. In addition, these studies have served to initiate research into the various components that make up these complex matrices and have attempted to identify the factors that are responsible for the beneficial health effects. The carotenoids, specifically beta-carotene, were identified as one of those major factors.[15]

However, the interpretation of these observational studies for carotenoids is difficult, for a number of reasons.[16] The effects of the food matrix and menstrual cycle stage were mentioned above. Additionally, individuals who consume a greater amount of fruits and vegetables often display other health conscious attributes, for example regular exercise, that could affect any conclusions. Perhaps most importantly, food delivers many nutrients and phytochemicals other than carotenoids that are active in the metabolism of the body. Notably, vitamins C and E are also strong antioxidants.

All of the reasons cited above combined to pave the way for large scale intervention trials testing the efficacy of beta-carotene. It is the results of these studies, especially the confusing results dealing with the incidence of lung cancer in smokers, that have garnered the majority of media attention in the past 2–3 years. I will very briefly review four different trials that provide a spectrum of results, then look at the intriguing but as yet incomplete efforts to unify them. A more detailed review has been given by Mayne.[16]

Alpha tocopherol beta-carotene (ATBC) trial

This study included 29 133 males in Finland aged 50–69 who were heavy smokers at entry (average 1 pack/day for 36 years).[17,18] The study was a two-by-two factorial randomized design with participants receiving either alpha-tocopherol at 50 mg/day, synthetic beta-carotene at 20 mg/day, a combination of these or a placebo for 5–8 years. The surprising result was that the group receiving the beta-carotene had an 18% higher incidence of lung cancer. However, as would have been predicted by the observational studies, those in the placebo group that had higher baseline serum levels of beta-carotene were associated with lower lung cancer incidence.[16]

Carotene and retinol efficacy trial (CARET)

This study included 18 314 asbestos workers and smokers.[19] This randomized, double-blinded, placebo-controlled trial provided the study group with 30 mg/day of synthetic beta-carotene and 25 000 IU/day of retinyl palmitate (vitamin A). This study was stopped 21 months early due to the ATBC results above and a 28% increased lung cancer incidence in the active intervention group. Thus, though the study was statistically inconclusive,[16] it basically replicated the ATBC trial.

Physician's health study

This study was a randomized, double-blind, placebo-controlled trial that enrolled 22 071 male physicians in the United States aged 40–84 years.[20] Out of this group 11% were current smokers and 39% were former smokers. The active intervention group received 50 mg/day of synthetic beta-carotene every other day for 12 years. The final results showed no statistical difference between the study and placebo groups, indicating neither benefit nor harm with respect to lung cancer even among smokers.

Linxian county, China intervention study

This study was carried out in an area of China that has a strikingly high incidence of esophageal and gastric cancer.[21] It included 30 000 men and women aged 40–69 years, approximately 30% of whom were smokers. A variety of different nutrient combinations were used including retinol plus zinc, riboflavin plus niacin, ascorbic acid plus molybdenum, and a combination of beta-carotene, selenium and alpha-tocopherol. In the group given the beta-carotene (15 mg), selenium yeast (50 µg) and vitamin E (30 mg as alpha-tocopherol) there was a 13% reduction in overall cancer deaths after 5 years and, though there was limited statistical power for lung cancer with only 31 total lung cancer deaths, a 45% decrease in lung cancer deaths.

There have been a number of different mechanisms suggested to explain the unexpected enhancement of lung cancer in some of these studies. One is the interaction of nutrients.[22] Fruits and vegetables contain many different compounds that serve as antioxidants. Some are known to work in concert and could be affected by very high doses of one compound such as beta-carotene. Another hypothesis is pro-oxidant activity of beta-carotene under the physiological state of a smoker's lungs. This mechanism seems biologically plausible but evidence in support of it is lacking at present.[23]

The implications of toxicity and dose is another intriguing interpretation of the discordant results given by Mayne.[23] In a broad range of observational studies, the optimal level of beta-carotene concentration in the plasma or serum (defined as the level in the quartile where overall cancer risk reduction was of the greatest magnitude) was > 15–20 µg/dL with lower levels associated with a greater overall cancer risk. In comparison, the intervention trials above had median post intervention blood carotenoid concentrations of: ATBC, 300 µg/dL; CARET, 210 µg/dL; PHS, 120 µg/dL; Linxian 85.5 µg/dL.

Comparing these numbers to the 'threshold' of 20 µg/dL and looking at the intervention study results described above suggests that populations with low beta-carotene status might benefit by increasing plasma concentrations, but very large increases of that single compound show little benefit and even potential harm in populations with increased lung cancer risk, for example smokers.

As has been the case with carotenoid research in general, this discussion has focused to this point on beta-carotene. However, there have been recent results suggesting a direct relationship between disease and other carotenoids. Notable among these are the relationships between lutein and eye disease and lycopene and prostate cancer. The macular region of the human retina has been shown to concentrate the carotenoid xanthophylls lutein and zeaxanthin.[24] The density of this selective concentration has been associated with the disease Age-Related Macular Degeneration, which is a progressive disease that is the leading cause of blindness in

Table 1. Approximate carotenoid composition (%) of commercially available natural sources of extracts or concentrates

	Beta-carotene	Alpha-carotene	Lutein/Zeaxanthin	Lycopene	Other carotenoids
Dunaliella	87	10	2	–	1
Palm	59	37	–	1	3
Marigold	–	–	100	–	–
Tomato	5	–	–	85	10

Note: the various geometric isomers are not distinguished.

adults over 40.[25] Hammond *et al.*[26] studied the relationship between visual sensitivity and pigment density in younger and older populations. They found a higher visual sensitivity in the young population that declined with age, consistent with the literature. However, in the older population visual sensitivity was directly related to the macular pigment density, suggesting that the macular pigment may retard age-related declines in visual function.

A study by Giovannucci *et al.*[27] was part of the Health Professional Follow-up Study. Through food-frequency questionnaire data they found that of the carotenoids, only lycopene showed a strong inverse relationship with risk of prostate cancer. The primary source of the lycopene was from processed tomato products. Their conclusions were that the findings suggest that intake of lycopene or other compounds in tomatoes may reduce prostate cancer risk.

Natural sources for carotenoids

The brief look at the relationship to disease suggests that there is more to the picture of carotenoids and human nutrition than just high doses of the all *trans* isomer of beta-carotene found in synthetic preparations. Much is yet to be learnt and many studies are under way. However, researchers repeatedly refer to the broad spectrum of carotenoids found in a high fruit and vegetable diet and in human blood as models to be followed for reduced risk of disease. Unfortunately, though, the synthetic chemical processes to produce this wide variety are difficult and expensive. Are there natural sources that could be used to form concentrates for supplement products?

Currently there are four different sources that are commercially available: the alga *Dunaliella*, palm fruit, marigold flowers (*Tagetes sp.*), and tomatoes. The approximate concentrations of the carotenoids that make up the concentrates commonly made from these sources are listed in Table 1.

It is obvious that any single natural source does not reflect the average to be found in a varied diet. In fact, it would be difficult to even determine a 'typical' diet because the availability of various fruits and vegetables varies greatly, both geographically and seasonally. The preferred approach at this time appears to be a mixture of the available natural sources. Such multicarotenoid supplements at reasonable doses, along with a spectrum of the other important nutritional components such as vitamins E and C, show promise for moving the diets of individuals unable or unwilling to consistently consume large quantities of fruits and vegetables.

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References

1. Straub O, Pfander H, eds. Key to carotenoids. Boston: Birkhauser Verlag, 1987.
2. Britton G, Liaaen-Jensen S, Pfander H *et al.* Carotenoids Volume 1A: Isolation and analysis. Boston: Birkhauser Verlag, 1995.
3. Olson JA, Krinsky NI. The colorful, fascinating world of the carotenoids: Important physiologic modulators (Introduction). FASEB J 1995; 9: 1547–1550.
4. Demmig-Adams B, Gilmore AM, Adam WW III. *In vivo* functions of carotenoids in higher plants. FASEB J 1996; 10: 403–412.
5. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: An evaluation of analytic data. J Am Diet Assoc 1993; 93: 284–296.
6. Ben-Amotz A, Fishler R. Analysis of carotenoids with emphasis on 9-*cis* beta-carotene in vegetables and fruits commonly consumed in Israel. Food Chem 1998; 62: 515–520.
7. Von Euler B, Von Euler H, Karrer P. Zur Biochemie der Cartinoide (in German). Helvetica Chimica Acta 1929; 12: 278–284.
8. Khachik F, Spangler C, Smith C Jr *et al.* Identification, quantification and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem 1997; 69: 1873–1881.
9. Parker RS. Absorption, metabolism and transport of carotenoids. FASEB J 1996; 10: 542–551.
10. de Pee S, West CE, Muhilal Karyadi D, Hautvast JGAJ. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 1995; 346: 75–81.
11. Forman MR, Johnson EJ, Lanza E *et al.* Effect of menstrual cycle phase on the concentration of individual carotenoids in lipoproteins of premenopausal women: A controlled dietary study. Am J Clin Nutr 1998; 67: 81–87.
12. Ben-Amotz A, Levy Y. Bioavailability of a natural isomer mixture compared with synthetic all-*trans* beta-carotene in human serum. Am J Clin Nutr 1996; 63: 729–734.
13. Krinsky NI. Actions of carotenoids in biological systems. Annu Rev Nutr 1993; 13: 561–587.
14. Krinsky NI, Wang XD, Tang G, Russel RM. Conversion of carotenoids to retinoids. In: Liurea MA, Packer L, eds. Retinoids progress in research and clinical applications. New York: Marcel Deker, 1993: 1–16.
15. Krinsky NI, Sies H, eds. Antioxidant vitamins and beta-carotene in disease prevention. Proceedings of a symposium held in Berlin, Germany, 10–12 October 1994. Am J Clin Nutr 1995; 62 (Number 6, Suppl.): S1299–1540.
16. Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 1996; 10: 690–701.
17. Albanes D, Heinonen OP, Taylor PR *et al.* Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: Effects of baseline characteristics and study compliance. J Natl Cancer Inst 1996; 88: 1560–1570.
18. Alpha-tocopherol beta-carotene cancer prevention study group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994; 330: 1029–1025.
19. Omenn GS, Goodman GE, Thornquist MD *et al.* Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial. J Natl Cancer Inst 1996; 88: 1550–1559.
20. Hennekens CH, Buring JE, Manson JE *et al.* Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996; 334: 1145–1149.

21. Blot WJ, Li J-Y, Taylor PR *et al*. Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 1993; 85: 1483–1491.
22. Mayne ST, Handelman GJ, Beecher G. Beta-carotene and lung cancer promotion in heavy smokers – a plausible relationship? J Natl Cancer Inst 1996; 88: 1513–1515.
23. Mayne ST. Beta-carotene, carotenoids and cancer prevention. In: DeVita VT Jr, Hellman S, Rosenberg, SA, eds. Principles and practice of oncology, 5th edition updates. Philadelphia: Lippincott-Raven Publishers 1998; in press.
24. Bone RA, Landrum JT, Taris SL. Preliminary identification of the human macular pigment. Vision Res 1985; 25: 1531.
25. Seddon JM, Ajani UA, Sperduto RD *et al*. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 1994; 272: 1413–1420.
26. Hammond BR Jr, Wooter BR, Snodderly DM. Preservation of visual sensitivity of older subjects: Association with macular pigment density. Invest Ophthalmol Visual Sci 1998; 39: 397–406.
27. Giovannucci E, Ascherio A, Rimm EB, Stampfer MG, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 1995; 87: 1767–1776.