Carotenoids
Volume 5: Nutrition and Health

Chapter 19

Editors' Assessment

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A. Introduction

Since the question: "Can dietary β-carotene materially affect cancer rates?" first surfaced in 1981 [1], a large research effort has been directed to trying to determine if this is indeed the case. Much of the work has been based on the premise that any effect is likely to involve antioxidant action (*Chapter 12*) or effects on cellular and molecular processes (*Chapter 11*). The participation of the immune response system (*Chapter 17*) and suggestions that effects attributed to carotenoids may be mediated *via* retinoids or other metabolites/breakdown products (*Chapter 18*) have also attracted much attention. A variety of experimental approaches have been used to investigate the relationship between carotenoids and the incidence of cancer (*Chapter 13*) or coronary heart disease (CHD) (*Chapter 14*), particularly human, animal and cell studies. With the eye (*Chapter 15*) and skin (*Chapter 16*), the situation is different. These tissues are exposed to high intensity light, that can lead to photodamage. Do carotenoids have any protective roles against this damage?

Some 20 years ago it was recommended that biological properties and effects of carotenoids should be divided into functions, actions and associations [2]. In the present context, a **function** is an essential role played by the carotenoid, under normal physiological conditions, in growth, development and maturation, and maintaining life. An effect that can be demonstrated after administration of a carotenoid is considered as an **action**. It may or may not have general physiological significance. The term '**association**' is used when a demonstrated effect is associated with the presence of a carotenoid but cannot be directly attributed to that carotenoid. This perceptive insight has helped to shape thinking about the subject since then.

There is no doubt that carotenoids are the main source of vitamin A for most people, particularly those most at risk from vitamin A deficiency (VAD) (*Chapter 9*). Whatever other effects of carotenoids may or may not be substantiated, carotenoids will always be of vital importance for their role as provitamin A. But what of the other actions? What do we really know about carotenoids and human health? Where is there still uncertainty? There is much information about the major topics such as cancer and coronary heart disease (CHD) but rarely any definite proof. There are tantalizing glimpses of other interesting observations that would merit further investigation. In all this we must be guided by knowledge of the properties of the carotenoids as they exist *in vivo*.

So much is published. We can read so much in the popular press, publicity literature and articles on the internet. We read and hear extravagant claims 'supported by scientific research'. It is possible to find scientific literature that will contain some selected material which, taken out of context, will appear to give such support to almost any claim, though this may be based on uncritical experimental design and/or uncritical interpretation of results. This can be very confusing and lead the inexperienced reader to take it all at face value. It is therefore the duty of the 'carotenoid world' to plot a way through this and give informed judgement and guidance. This is what the authors in this *Volume* were asked to do and have done so well. They have given reasoned judgement and address some frequently asked questions. To a large extent, however, each chapter is a detailed account of one particular topic. The editors now attempt to put all this together to build a picture of where the subject stands today and what the future may hold.

We do not judge if studies and interpretations are good or bad. Our authors have done this when researching their chapters. Rather, we ask questions and raise points and recurring themes which readers should take into account when forming their own judgement and evaluation of published material.

B. From Food to Tissues

1. Sources, bioavailability and conversion

Apart from supplements (*Chapters 4* and *5*), we obtain our carotenoids from our food, primarily vegetables and fruit (*Chapter 3*). We therefore need to know what carotenoids are present and how much. Following the complex processes of digestion, absorption, transport and deposition, ingested carotenoids can be found in blood and other tissues and organs. Powerful methods are now available for analysing carotenoids in food, and in blood and body tissues (*Chapter 2*). For routine analysis, the use of HPLC is widespread but, without proper knowledge of the principles of separation, and without rigorous identification and peak assignment, this can lead to misleading information. Though upwards of 100 different carotenoids are present in all food sources in a varied diet when food is in good supply, few

carotenoids are present in blood and tissues and those for which associations with health and biological activity have been studied are fewer still. Generally, these are the only ones that are included in the food composition tables. Quantitative analysis by HPLC is extremely precise but requires careful attention to sample preparation and critical appraisal of the level of precision that is realistic and acceptable. Also, analytical results recorded are for a particular sample grown in a particular place under particular, often optimized conditions, so the quantitative figures often recorded in food composition tables at high levels of precision cannot be expected to apply universally, when there is such variability between samples. If this is not recognized, food composition tables can be misleading. But, as general guides to what foods may be good sources of total or particular carotenoids, they are very valuable. There is also a need for a simple, inexpensive and reliable method for rapid basic analysis of, for example, the food that is actually being eaten, even in remote areas where facilities for laboratory analysis do not exist.

It is once the carotenoids are in the human body that the major uncertainty begins. Many factors affect bioavailability (*Chapter 7*). Apart from the great variability between individuals, a major factor is release from the food structural matrix. Bioavailability from oils or supplements is much better. We can make generalizations but these do not necessarily relate to any particular individual or food source/product.

A wide range of precise numerical factors for the conversion of provitamin A carotenoids to vitamin A have been reported in different studies (*Chapter 8*). Again there is much variability among individuals, so concentrating on the numbers can divert attention from the important questions about what factors strongly regulate or influence the conversion, such as the vitamin A status of the subject, the dose of provitamin given, the form and formulation presented, the food structure, and methods of cooking and processing. The numbers do, however, give us a picture and information on which to base guidance on important points and take steps to optimize the conversion. The accelerator mass spectrometry (AMS) technique [3] provides an extremely sensitive means of analysing isotopic tracers at a very low level and may prove invaluable for bioavailabilty/conversion studies.

2. Variability between individuals

In all questions about bioavailability and conversion, the great variability between individuals is a large and uncontrollable factor. There is great variability between individuals, even between members of the same population, community or family. It is recognized that there are 'responders' and 'non-responders' or 'low-responders' in terms of uptake, deposition and conversion of carotenoids, leading to great variability in carotenoid concentrations in blood and tissues following similar intake. Mean values for a population sample are likely to be derived from a wide span of values and may be of limited value. It may be the personal parameters of each individual that are important and there may be an optimal beneficial level for each individual. Below this, the risk of serious disease increases; above it, harmful effects

become a possibility. Working on the basis of mean values could, therefore, be risky. The same value could correspond to a low carotenoid status in one individual but a high status in another. With the mapping of the human genome, new technologies of molecular biology and molecular genetics hold the key to solving these mysteries. An important example is understanding the basis of 'responders' and 'non-responders' by identifying the genetic and other factors that determine how efficiently an individual absorbs and stores carotenoids and converts them into vitamin A. This would open the door to real progress in defining the needs of individuals and populations. Recent work has revealed that genetic variations (single nucleotide polymorphisms, SNPs) can have a profound influence on the efficiency of the β-carotene-cleaving enzymes [4].

C. Carotenoids and Major Diseases: Practical Concerns and General Points

Chemistry and physics generally give definite answers. With biology this is often not so, and certainly not in the context of human health. Progress relies on building up a body of information, often based on statistical analysis and probability. The large variability between individuals is problematic. The biological cell is a complex system, multicellular organisms and their organs and tissues even more so. The complexity of the human body is almost incomprehensible. When looking at one factor we must always be aware that something completely different may be happening somewhere else or in another functional system. It is important not to think of one disease or effect in isolation. For example, when considering what may be recommended for skin health, it is necessary to consider what the consequences may be for cancer, CHD *etc*.

There are many reports of associations between higher carotenoid intake and reduced risk of major diseases such as cancer and CHD, but generally there is no proof of direct involvement and many questions remain. None of the experimental approaches on its own provides conclusive proof about whether carotenoids do have any effects and benefits for a real person under normal physiological conditions. But when they are taken together, indications begin to emerge. Here we draw attention to some aspects of experimental procedures that readers should bear in mind when evaluating results.

1. Human studies

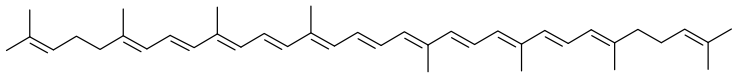
Epidemiological surveys (*Chapter 10*), based upon reported normal food intake, may identify associations between high daily intake of total carotenoids or a particular carotenoid and reduced risk of serious degenerative diseases such as cancer and coronary heart disease. But there are particular areas of uncertainty. It is very difficult to distinguish whether any effect seen is due to the carotenoids or to the foods in which the carotenoids are concentrated. When carotenoids are given in pure form as supplements in intervention trials (*Chapter 10*), they

introduce other uncertainties about the formulations and especially the doses administered, which are usually much higher than those obtained from the diet. Even if the doses given are comparable to levels in food, they have greater bioavailability and thus provide larger amounts. On the other hand, the slow release and absorption of the carotenoids during the digestion of food could also be a factor.

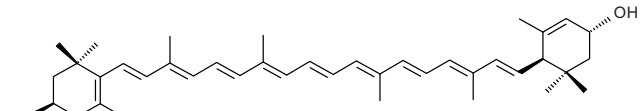
a) Are effects due to carotenoids or to food?

There is no doubt that there are associations between high carotenoid intake from natural food and hence higher concentration of carotenoids in blood and tissues, and reduced risk of serious diseases and conditions, especially cancer and CHD. But this does not prove that any protective effect is due to the carotenoid. The presence of a high level of carotenoid may simply be an indicator of a healthy diet rich in fruit and vegetables. Some other factor could be the biologically active principle; possibilities include fibre, other phytochemicals and antioxidants.

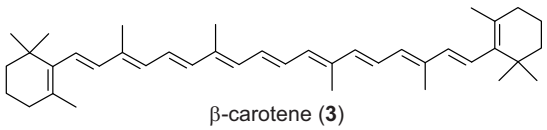
Advances in plant breeding and GM (*Chapter 6*) provide a possible approach to this problem. Red or yellow strains of carrots have been produced that accumulate lycopene (**31**) or lutein (**133**) instead of the β-carotene (**3**) and α-carotene (**7**) in familiar orange carrots.



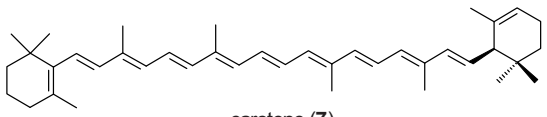
lycopene (**31**)



lutein (**133**)

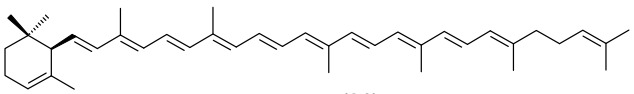


β-carotene (**3**)



α-carotene (**7**)

Similarly, tomato strains that accumulate high concentrations of β-carotene or δ-carotene (**21**) in place of the usual lycopene, or even no carotenoid at all, are available. Comparison of feeding trials with carrots or tomatoes that provide different carotenoids, or between strains of the different sources that provide the same carotenoid, *e.g.* lycopene or β-carotene, may allow effects of carotenoids and the food material itself to be distinguished.



δ-carotene (**21**)

b) Biomarkers

It is important to have reliable biomarkers of carotenoid status. Reported, often retrospective, food intake patterns carry a degree of uncertainty and cannot take into account factors such as variation in bioavailability among individuals. An analytical biomarker such as blood or tissue carotenoid concentration should be more reliable, but a non-invasive method that does not require the taking of blood samples or tissue biopsies would be ideal. Some recent developments are worthy of note. Non-invasive methods, *e.g.* resonance Raman spectroscopic or reflectance photometric methods, for rapid analysis of carotenoids in the skin *etc*. as a biomarker of carotenoid status look promising, but further rigorous validation is necessary before results can be accepted with complete confidence and the methods can be applied extensively.

2. Cell cultures

Carotenoids can be shown to influence cell signalling at both protein and transcriptional levels in cell cultures *in vitro* (*Chapter 11*), but can the findings be related to the complex intact biological system that is the human body?

At high concentration, carotenoids and almost any other substance can be shown to have some effect. But that does not mean that this is relevant *in vivo* and at concentrations thay may be present in the cell under normal conditions.

3. Animal models

Research on effects of carotenoids in laboratory animals, mostly with rats, mice and ferrets, has generated much information. But these animals differ from humans in important biological features, so the data obtained may well not be applicable to the human. The most scientifically supportable solution would be to use our closest relatives, other primates, as models for experimental studies, but the cost of extensive trials would be prohibitive and many people have moral concerns about using primates in such a way.

4. High dose, low dose and balance

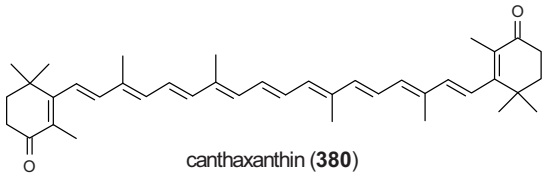
Much of the experimental work to study effects of carotenoids has used non-physiological doses or has applied carotenoids to cell cultures *etc*. in higher than physiological concentrations. The natural amounts of carotenoids in food are around 5 mg per day, whereas human trials typically use 20-100 mg/day, and equivalent levels are used in animal studies. It is common that beneficial associations are seen with carotenoids provided at the normal levels in food or with low levels as supplements, but adverse effects may be seen with the high doses. The unnaturally high pharmacological doses may be treated by the body as foreign substances, triggering detoxication mechanisms, including cytochrome P450 enzymes.

Prolonged, repeated intake of small amounts may be more effective than a single large dose. Good intake throughout life, starting at an early age, may be better than taking large doses as supplements later in life.

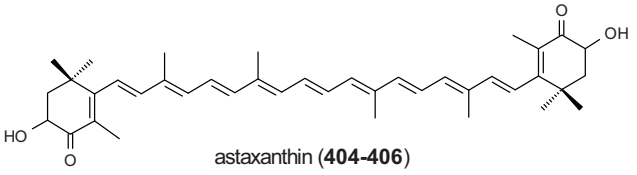
It is important to maintain a balance in cells and within the body, *e.g*. among carotenoids and with other factors, *e.g*. antioxidant vitamins C and E. High-dose supplements disturb the balance and may lead to unexpected and totally different results and consequences.

5. Safety and toxicity

The results of the ATBC and CARET trials from which it was concluded that daily high-dose β-carotene supplements increase the risk of lung cancer in heavy smokers have received much publicity. In non-smokers there is no evidence that high intake of carotene, either as supplements or from food, such as large amounts of carrots, has any serious adverse effect. The carotenodermia sometimes seen appears not to be harmful and the orange colour soon disappears when the excessive carotene consumption is discontinued. Despite much discussion, no recommended safe upper limit for carotene intake has been agreed. The taking of high-dose supplements of β-carotene by the general population is not recommended, though there is no recommendation that the use of such supplements for treatment of the photosensitivity disorder erythropoietic protoporphyria has dangerous consequences or should be discontinued.



canthaxanthin (**380**)



astaxanthin (**404-406**)

The practice of using oral canthaxanthin (**380**) capsules to give a tanned appearance of the skin has been discontinued because of cases in which microcrystalline deposits of canthaxanthin were seen in the eye. This appeared to have no long-term consequences and was rapidly reversed when canthaxanthin was no longer given.

Astaxanthin (**404-406**) supplements are now being promoted and are reported to have many beneficial effects. At normal dietary levels, astaxanthin is not detected in blood. With high dose supplements it may be, and it has been found to be taken up directly into erythrocytes. The consequences, good or bad, of this supplementation are unclear and must be evaluated.

No safety issues have been raised about other carotenoids, notably those that are now available and taken by some as dietary supplements, especially lutein, lycopene and astaxanthin. Equally, however, there is no direct evidence to prove that these compounds are completely safe. Knowledge of the properties of lycopene as a carotenoid that is easily oxidized and can have strong pro-oxidant properties suggests that, if trials with lycopene similar to the ATBC trials were undertaken, the adverse effects seen in smokers could be worse than with β-carotene.

Carotenoid supplements are subject to food legislation (*Chapter 4*). This seems reasonable if they do not lead to a total intake much greater than the normal dietary level that can be obtained from food. The high supplementary levels represent an unnatural situation, and the long-term effects are generally not known. It is not unreasonable that the use of carotenoids at these high pharmacological doses should be subject to the same stringent testing that is applied to pharmaceutical products.

6. Geometrical isomers

Now that modern analytical methods, especially HPLC, can readily detect geometrical isomers of carotenoids, and *Z* isomers are routinely found in food, blood and tissues, the question of whether they have any biological significance arises frequently. Some of the findings seem strange. For example, why should there be such great differences in the proportion of *Z* isomers in blood and tissues compared to the food sources? Various possibilities, such as enzymic conversion in the tissues have been suggested to explain this. Usually the only factor considered is the interconversion of isomers. This is always an equilibrium and should always lead to the same equilibrium mixture. Conditions will determine how long it takes for the equilibrium to be reached. But the *E/Z* isomerization is not the only equilibrium to consider. It is linked to other equilibria and mass action effects. All-*E* and *Z* isomers have different shapes and solubilities. All-*E* isomers aggregate and crystallize easily. Only the relatively small proportion that is in the monomeric form is available to participate in the isomerization equilibrium. Once formed, the *Z* isomers remain in solution, do not aggregate to any appreciable extent, and can be taken up and transported. The all-*E* isomer is more likely to re-aggregate, so the amount of this available to be taken up is small.

So, overall, the proportion of *Z* isomers that can be transported and deposited in tissues increases.

7. Natural *versus* synthetic

For some years there has been a public or at least media perception that 'natural is preferable to synthetic'. But a debate of this kind should be based on facts and not driven by emotion and deeply entrenched positions. If a carotenoid or other compound is pure, then natural and synthetic samples are chemically identical. Their biological actions must also be identical, provided they are in the same physical state and formulation. A natural extract rich in a particular carotenoid is not comparable to the pure form, natural or synthetic, because of the presence in the extract of many other components, any of which could have biological activity. There are, for example, reports that describe some biological effect of tomato-based products or tomato oleoresin and then attribute this effect to lycopene. The reader should look out for this in publications.

Production from natural sources is also generally considered to be more 'environmentally friendly' than industrial chemical synthesis, but is this now necessarily so? In industrial chemical synthesis there are strict controls on emissions, waste recycling or disposal *etc*., so damage to the environment is minimized. Similar controls should be in place for industrial-scale extraction from natural materials, with its large requirement for solvents. The environmental cost of production of natural sources must also be considered. Production of a single crop in large areas of monoculture may involve a high cost in terms of the destruction of natural ecosystems or may use land and resources that would otherwise be used for food production.

The arguments are not simple, however. It is often said that the spread of oil palm plantations has come at the cost of large scale destruction of natural forests. But the main objective of the vast oil palm industry is to produce chemicals for soaps and detergents, and especially biodiesel. Production of the nutritionally rich carotene products is only a very minor part of the palm oil operation, and provides a useful and valuable product from what would otherwise be waste material from the major operations.

The use of other waste products would also seem to be an ideal goal, *e.g*. tomato skins for lycopene production, and would reduce the pressure for demand to be met from natural wild resources or additional extensive cultivation.

It is likely that new potentially valuable sources of carotenoids will be discovered, especially in remote places and these may be part of specialized ecosystems. As with so many natural resources, their uncontrolled exploitation could lead to serious environmental damage.

It is right that this debate should continue openly but should be based on the facts. Each case should be considered on its merits, and all aspects should be taken into account.

D. How Might the Effects be Mediated?

1. *Via* antioxidant action

Harmful reactive oxygen species and free radicals are continuously produced in the body during normal cellular functioning and are introduced from exogenous sources. Damage caused by these is associated with aging and with the incidence and progression of serious diseases including cancer, CHD, age-related macular degeneration and neurodegenerative conditions.

Although the body has a battery of defences against this (enzymes and endogenous antioxidants), it is widely believed that dietary supplementation with antioxidants can be a part of a protective strategy to minimize the oxidative damage, especially in the elderly and other vulnerable populations. But can carotenoids be counted among the effective antioxidants *in vivo*? Since the concept was first raised in 1984, much of the research on carotenoids and human health has been driven by the prospect that carotenoids may be among the group of antioxidants in fruits and vegetables that help to prevent damage caused by oxidizing free radicals. It is enlightening to compare the conclusions given in that paper [5] with the many other interpretations published by other reviewers and commentators.

Carotenoids have been shown to have antioxidant activity *in vitro* at physiological oxygen tensions. Most work has been done with model systems with oxidizing free radicals generated artificially from azo-initiators. Assays have usually addressed parameters of lipid peroxidation, most commonly the TBARS reaction, the reliability of which in the presence of carotenoids has been questioned; carotenoid oxidation products can give a positive reaction. Experimental design and control must be rigorous. Purity of the carotenoid tested is essential; if it is not free from peroxides, completely different results can be obtained. Comparison between different studies and different systems is, therefore, fraught with difficulty.

Model and non-biological studies of antioxidant effects are usually undertaken under conditions that do not resemble those *in vivo*. To demonstrate antioxidant activity in a natural system *in vivo* is extremely difficult; the system is so complex, conditions are difficult to control and are not uniform within the system, natural carotenoid concentrations are low and many interactions with other substances are possible.

There is, however, evidence that a synergistic/cooperative interaction between carotenoids and antioxidants such as tocopherols, ascorbic acid, and flavonoids, may play a role in the biological antioxidant network. This does not prove that carotenoids are antioxidants *in vivo*, however. It may be that the antioxidants protect the carotenoid and prevent the formation of pro-oxidant carotenoid peroxides and peroxy radicals, or regulate the formation of oxidative breakdown products that could have biological actions?

2. *Via* metabolites

A question that frequently arises is whether any effect attributed to a carotenoid is in fact due to a metabolite/breakdown product rather than to the intact carotenoid itself (*Chapter 18*). For any carotenoid, there are many products, as complex mixtures. Any one, or combination, could have some biological activity. The roles of vitamin A and retinoic acid are well known but evidence is accumulating to suggest that other non-retinoid breakdown products may be biologically active. Some possibilities that have been suggested are:

(i) β-Carotene cleavage products of different chain length or cleavage products of other carotenoids, bearing structural features (end group or chain) in common with retinoids, could act as retinoid agonists or antagonists.

(ii) Many carotenoid oxidation products have reactive α,β-unsaturated aldehyde structures. By binding to side-chain amino groups or, for dialdehydes, cross-linking, these could modify properties and activity of enzymes or other proteins.

(iii) The biological activity of many compounds is based on size, shape and position of functional groups. Although otherwise structurally unrelated, a carotenoid breakdown product could have the right topography to mimic some other molecule, *e.g.* vitamin D, hormones, and act as an agonist or antagonist.

3. *Via* the immune system

Carotenoid status is associated with immunocompetence (*Chapter 17*). Effects of several different carotenoids on various parameters of the human immune response system have been demonstrated, mostly in experiments *in vitro*. It is known that vitamin A strongly influences the immune system. When this is extended to provitamin A carotenoids, it is always possible that any action is *via* vitamin A. Effects are also seen, however, with some non-provitamin A carotenoids, especially lutein and astaxanthin. The question of whether antioxidant effects, or action *via* non-retinoid carotenoid metabolites may be involved in any such effects has been raised. This potentially interesting topic merits further careful investigation.

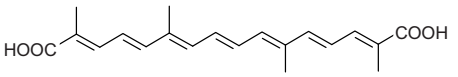
E. Reports of Other Health Effects

Possibilities of other effects of carotenoids on other aspects of health have been proposed. Most have not been pursued, but some do look interesting, though much more work is needed to substantiate any of these suggestions. The work is so far not extensive enough to warrant specific coverage in this *Volume*, but the topics are worth further consideration.

1. Water-soluble carotenoids

Carotenoids are usually not soluble in water and are therefore located in a hydrophobic environment *in vivo*. Carotenoid dicarboxylic acids, however, have appreciable solubility in water, allowing them to remain in an aqueous environment in cells or fluids.

Effects of the carotenoid dicarboxylic acid crocetin (**538**) and its disodium salt on haemorrhagic shock and wound trauma have been reported and attributed to increasing pulmonary oxygen flow to tissues [6]. It is proposed that the dipolar structure and its associated water shell impart special properties to the molecule. It would be interesting to know if similar effects could be found with carotenoid dicarboxylic acids of other chain lengths.



crocetin (**538**)

2. Bone health

Bone health is the result of two opposing processes, bone formation and bone resorption. These are regulated by various factors, notably hormones and vitamin D. β-Carotene, lycopene and other carotenoids have been associated with a role in supporting bone formation *via* increasing levels of bone-forming proteins [7]. This would constitute a benefit of carotenoids against brittle bones in the elderly. In contrast, high intake of vitamin A is associated with loss of bone mineral density and risk of osteoporosis (*Chapter 9*).

3. Metabolism and mitochondria

In recent years there are increasing suggestions that carotenoids (most experimental work has been with astaxanthin) may help to offset the imbalance known as 'metabolic syndrome' by increasing mitochondrial efficiency, energy metabolism and especially fat metabolism, thereby helping to reduce obesity and enhance athletic performance [8]. In intense and prolonged exercise, the level of metabolism in mitochondria in the muscles is high and oxidative stress greatly increases. Also, according to the 'mitochondrial theory of aging', oxidative damage, to DNA, protein and lipids, accumulates in the mitochondria over the lifetime of the organism. Mitochondrial dysfunction leads to many consequences, including pro-oxidative changes in redox homeostasis and efflux of mitochondrial components, notably cytochrome *c*. This has been linked to effects on signalling mechanisms, impairment of the immune response and neurodegenerative conditions (*Chapters 11* and *17*). The indications are interesting enough to merit further rigorous study, which should be extended to other carotenoids.

F. Final Comments: The Big Questions

(i) "Apart from their clear function as dietary precursors of vitamin A, do carotenoids have other functions or actions that are beneficial to human health?"

A number of associations have been reported between higher concentrations of carotenoids in the blood and reduced risk of serious diseases such as cancer and CHD. Also actions of carotenoids on various cellular and molecular processes have been demonstrated in cells in culture. Intervention trials to try to show a direct relationship between administered pure carotenoid intake and disease risk have been less informative; conflicting results are common.

(ii) "Are carotenoids important antioxidants *in vivo*?"

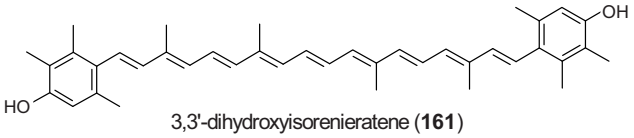
Although it is clear that carotenoids can serve as antioxidants *in vitro*, there is no unequivocal evidence for their functioning in this way *in vivo*. Indeed, carotenoid concentrations are low compared to those of recognized antioxidants such as vitamins C and E, so activity as a general antioxidant seems unlikely, but some specialized action in a particular sub-cellular environment such as a membrane, or in particular tissues, cannot be ruled out. All aspects must be considered. It is suggested, for example, that protection of LDL by lycopene as an antioxidant may be a factor in reducing risk of CHD. If this is looked at carefully, calculations show that, on average, there is only about one carotenoid molecule per four LDL particles. This does not seem compatible with a major antioxidant role.

(iii) "Do carotenoids help to provide protection and reduce risks of serious degenerative diseases?"

The honest answer to this and to most of the questions about the roles of carotenoids in human health is that we simply do not know. After many years of extensive and painstaking research, definite answers and unequivocal evidence are hard to find, though there is much circumstantial and indicative evidence. Much time and many resources have been spent chasing one popular idea to the exclusion of others or simply repeating experiments with one carotenoid after another. Would we now be better informed and have a better understanding, for example, if some of the massive research effort devoted to elucidating the proposed role of carotenoids as antioxidants had been channelled in other directions?

(iv) "Might some other carotenoids be important for health?"

Few of the hundreds of natural carotenoids have been studied for effects on human health. It is always possible that some, even ones that are not components of a normal diet, could have biological activity, though the question of safety and toxicity must be observed. One interesting structure is 3,3′-dihydroxyisorenieratene (**161**) which combines structural elements of carotenoids and tocopherol.



3,3'-dihydroxyisorenieratene (**161**)

(v) "Is it good for health to take carotenoid supplements?"

The bulk of evidence and the consensus of opinion supports the conclusion that intake of carotenoids at the levels obtained in a normal balanced diet is safe and may be beneficial, but the safety of larger intake, especially the high doses often taken as supplements is, at best, not proven. The reader must judge from the evidence. There is much uncertainty and it would not be wise or responsible of us to advocate the routine use of carotenoid supplements at levels above the normal dietary level of 5 mg/day.

We make no recommendation either way; personally, however, we are not convinced to take supplements but do try to maintain a good supply of carotenoids from our normal diet.

(vi) Finally, have we any advice for the researcher taking up the exciting challenges in this field?

Evaluate the literature carefully and critically. Look at experimental design and make your own interpretation. Don't rely only on interpretation by the author or by other reviewers or commentators. Make sure that any ideas and conclusions are compatible with the properties of the carotenoid as it exists in its natural state and surroundings. So enjoy it and:

Above all, be open-minded and expect the unexpected!!

References

[1] R. Peto, R. Doll, J. D. Buckley and M. B. Sporn, *Nature*, **290**, 201 (1981).

[2] J. A. Olson, in *Modern Nutrition in Health and Disease* (ed. M. E. Shils, J. A. Olson, A. C. Ross and M. Shike), p. 521, Saunders, Philadelphia (1998).

[3] R. S. Dueker, Y. Lin, A. B. Buchholz, P. D. Schneider, M. W. Lamé, H. J. Segall, J. S. Vogel and A. J. Clifford, *J. Lipid Res*., **41**, 1790 (2000).

[4] F. Tourniaire, W. Leung, C. Méplan, A.-M. Minihane, S. Hessel, J. von Lintig, J. Flint, H. Gilbert, J. Hesketh and G. Lietz, *Carotenoid Sci*., **12**, 57 (2008).

[5] G. W. Burton and K. U. Ingold, *Science*, **224**, 569 (1984).

[6] L. J. Giassi, A. K. Poynter and J. L. Gainer, *Shock*, **18**, 585 (2002).

[7] S. Sahni, M. T. Hannan, J. Blumberg, L. A. Cupples, D. P. Kiel and K. L. Tucker, *Am. J. Clin. Nutr*., **89**, 416 (2009).

[8] M. Ishikara, *Carotenoid Sci*., **12**, 3 (2008).