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Review

Photochemical and Photophysical Properties of Carotenoids and Reactive Oxygen Species: Contradictions Relating to Skin and Vision

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Abstract: Molecular mechanisms based on photo-physical processes involving dietary carotenoids, their radicals, and the role of oxygen are discussed and used to suggest explanations of the poorly understood and often contradictory results related to mainly skin and vision. Differing and conflicting efficiencies of singlet oxygen reactions with carotenoids of biological importance are discussed in environments from 'simple' organic solvents to single He La cells. A range of free radical reactions with carotenoids, and the corresponding radicals of the carotenoids themselves, are compared and used to explain the switch from beneficial to deleterious processes involving dietary carotenoids and to unravel their differing functions; of particular interest is a possible role for vitamin C.

Keywords: carotenoids; β-carotene; lycopene; zeaxanthin; lutein; astaxanthin; reactive oxygen species; reactive nitrogen species; singlet oxygen

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1. Introduction

Many studies involve so-called reactive oxygen species (ROS) and reactive nitrogen species (RNS), but without consideration of the individual radical and non-radical components of such complex mixtures of reactive species. A major aspect of this review is that we discuss the individual components of ROS (as well as RNS) in an attempt to understand the underlying photophysical molecular mechanisms and, hence, to explain apparent contradictions with respect to the dietary carotenoids.

Carotenoids can be hydrocarbons such as β-carotene (β-car) and lycopene (lyc), or xanthophylls (oxygen containing carotenoids) such as zeaxanthin (zea), its stereo-isomer meso-zeaxanthin (mzea), lutein (lut), canthaxanthin (can), and astaxanthin (ast), and occur widely in nature, but are only present in humans, and most animals, via their diet. Of the over 700 or so carotenoids and xanthophylls known, only about 40 accumulate in humans.

However, they play a critical role in vision, the skin, and disease prevention in general, and yet they can also lead to deleterious health damage. The apparent contradictions, in terms of carotenoid photochemical and photophysical properties, are examined in this review based on the underlying molecular mechanisms. Both unexpected consequences of the role of carotenoids and contradictory views on the reactions of carotenoids are discussed. Of course, there are important health related non-photochemical processes of dietary carotenoids (such as the effects of carotenoid loadings in different environments, the role of carotenoid metabolites, gap junctions, and chemoprotection), but these are not considered in this review. There are several recent reviews of the reactions of ROS with carotenoids [1–3], but in this review we emphasise contradictory and possibly surprising or poorly understood aspects of such processes.

2. Singlet Oxygen

One of the earliest studies of a specific ROS concerned singlet oxygen and carotenoids. As is well-known, all carotenoids with 11 conjugated double bonds (n) quench singlet oxygen very efficiently with rate constants near the diffusional rate in organic solvents, but this efficiency is significantly reduced with n only 10 (or less). The most important example concerns lutein (lut, n = 10). As will be discussed below, lut is a key macular pigment along with zea and mzea (n = 11). So, from a photophysical aspect, it is of interest that the macular contains not only zea and mzea but also lut, even though it is a less efficient singlet oxygen quencher.

Another aspect of interest concerns a comparison of lyc with other n = 11 dietary carotenoids, especially β-car in organic solvents. In early work [4], Di Mascio and co-workers showed lyc to be over twice as efficient at quenching singlet oxygen than β-car (3.1×10^{10} $M^{-1}s^{-1}$compared to 1.4×10^{10} $M^{-1}s^{-1}$). However, Conn et al. [5] showed there was a much smaller difference in benzene and toluene with average values of 1.80×10^{10} $M^{-1}s^{-1}$ compared to 1.35×10^{10} $M^{-1}s^{-1}$. Indeed, this work compared six solvents including the complex mixture used by Di Mascio and could not replicate their results, thus showing that there is not a substantially increased quenching efficiency of singlet oxygen by lycopene compared to other carotenoids. While both these sets of older data refer to environments far removed from those of biological interest, the DiMascio result is still frequently quoted in the recent biological literature, for example [6–8]. There is no clear reason for these different results; certainly lyc is a particularly insoluble carotenoid in polar solvents, and this could lead to experimental difficulties. The small increase in singlet oxygen quenching efficiency by lyc compared to β-car is probably related to steric effects. There is a loss of planarity in β-car and other C_{40} carotenoids and xanthophylls with terminal six membered rings. This creates twisting of the rings due to steric hindrance, leading to an effective reduction in the conjugated chain length, which is not present in lyc with no terminal rings. Many other carotenoids have also been studied with regard to their singlet oxygen quenching ability in organic solvents, and it has been shown that quenching ability increases with increasing number of conjugated double bonds.

This efficiency of singlet oxygen quenching by carotenoids can be reduced by several factors—aggregation, isomerization and, as noted above, the number (n) of conjugated double bonds in the carotenoid. The effects of aggregation may well be important in some biological environments, and we thank the referee for drawing our attention to the importance of the carotenoid site with respect to the car photophysical properties in the real-life situation.

Studies in micro-heterogeneous environments such as micelles, liposomes, and cells are well reviewed recently, see for example [3,9]. One aspect which may contribute to a contradiction with clinical results (see below on porphyria) concerns studies where the singlet oxygen is generated either in the organic/lipid phase, where hydrocarbon carotenoids, including β-car, accumulate, or in the aqueous phase via a water-soluble photosensitiser, where there is no β-car. Importantly, in these photophysical liposomal studies, the quenching efficiency of the carotenoid was independent of the site of singlet oxygen generation (i.e., whether the singlet oxygen was generated by a water or lipid soluble photosensitiser) [10,11]. Additionally, in multilamellar liposomes, lyc has been observed to be a slower quencher of singlet oxygen than β-car (0.8×10^{10} $M^{-1}s^{-1}$ compared to 1.9×10^{10} $M^{-1}s^{-1}$; Cantrell & Truscott, unpublished result).

For oxygen containing carotenoids (xanthophylls), which are important in macular protection (discussed below), there is extensive carotenoid aggregation and, as a result, a huge reduction in the singlet oxygen quenching efficiency. However, such aggregation may well lead to a change in the lipid structure and, for example, increase fluidity, which will add to the complexity of understanding any anti- or pro-oxidative effect [12].

A study by Ogilby and co-workers [13] using microscopy to observe the time-resolved singlet oxygen luminescence in single HeLa cells has shown no change in the lifetime of intracellular singlet oxygen in the presence of β-car, even in D_2O where the singlet

oxygen lifetime is significantly lengthened (15–40 μs) compared to water (about 3 μs). Thus, these researchers suggest that the protective effects of β-car observed in their cell environment may be due to radical trapping and not direct singlet oxygen quenching, and they propose this is due to a very low diffusion rate within the high viscosity intracellular environment. A related result [14] showed that in reverse micelles singlet oxygen is removed, i.e., quenched, forming carotenoid endoperoxides. Interestingly, in view of the singlet oxygen lifetime studies comparing β-car and lyc discussed above, these workers showed this process is twice as efficient for lyc as β-car. In addition, other cellular studies have shown differing results to those of Ogilby et al. Thus, carotenoids have been shown to efficiently quench singlet oxygen in isolated photosystem II (PSII) reaction centres [15] and can also protect ex vivo lymphocytes from singlet oxygen-induced damage [16,17]. This result for PSII may well be due to the 'forced' proximity of the singlet oxygen generating system to the carotenoid.

Even though the results from Ogilby were somewhat surprising, far more surprising is the recent postulate from Moskalenko and co-workers [18] arising from photosynthetic bacteria studies. This work shows that carotenoids do not protect bacteriochlorophylls in isolated light-harvesting LH2 complexes of photosynthetic bacteria. However, they also show that a decrease in the amount of carotenoids in the bacterial complexes leads to more efficient protection of bacteriochlorophyll from singlet oxygen oxidation. This observation leads to the speculation that carotenoids, upon excitation with blue light, are capable of generating singlet oxygen. We suggest this must be thoroughly confirmed before being accepted—the ultra-short lifetimes of the carotene excited states would seem to preclude such a process.

3. Radicals

The most important free radicals of biological interest are probably the hydroxyl radical ($OH^{\bullet}$), superoxide radical anions ($O_2^{\bullet -}$), which are produced in the body during normal metabolic processes; the environmentally important nitrogen dioxide ($NO_2^{\bullet}$) and a range of lipid radicals ($R^{\bullet}$).Peroxyl lipid species ($ROO^{\bullet}$) are also generated in vivo upon oxygen addition to $R^{\bullet}$. Photosensitisation reactions in vivo (e.g., in the skin) are initiated by formation of a triplet state of a photosensitiser (e.g., porphyrins and riboflavin). Electron or hydrogen atom transfer to this triplet state from an organic substrate (RH) produces a carbon-centred radical ($R^{\bullet}$), which, in the presence of molecular oxygen, will produce a peroxyl radical ($ROO^{\bullet}$). A chain reaction can then follow, thus propagating the oxidation. It is such peroxyl radicals that may well be related to the important switch from anti- to pro-oxidation by carotenoids as a function of oxygen concentration, as discussed below. Of course, other radicals are also of some interest, such as oxygen-sulphur-based systems, which are environmental pollutants, but these are not discussed in this review.

Hydroxyl radicals are one of the most important radicals produced in vivo, e.g., via the metal-ion catalysed Haber–Weiss reaction. They are extremely oxidising, having a reduction potential of 2.31 volts vs SHE at pH 7, which can be even higher in acidic environments [19,20]. As such, they are able to oxidise many biologically relevant compounds, but they can also react via hydrogen abstraction, for example with amino acids, and can add across carbon-carbon double bonds, as seen with purines.

Superoxide is not a very reactive radical species in aqueous environments and normally acts as very mild reductant, although it can also act as an oxidant and is highly reactive in hydrophobic environments. It may seem contradictory, but despite this low reactivity in aqueous environments, superoxide radicals are important biologically since they are perpetually generated in normal metabolism and produced in phagocytic cells as a method to inactivate both bacteria and viruses [19]. When cells are activated for phagocytosis there is a large, at least 10-fold, increase in oxygen consumption followed by a reduction of this oxygen to superoxide catalysed by plasma membrane-bound NADPH oxidase [21]. In recent work, using gamma radiation, we showed significant cell membrane damage to human lymphoid cells by superoxide, as well as hydroxyl radicals, with an oxygen

concentration effect of carotenoid protection observed [22,23]. Finally, of course, superoxide dismutase (SOD), is indispensable as an antioxidant, removing the superoxide.

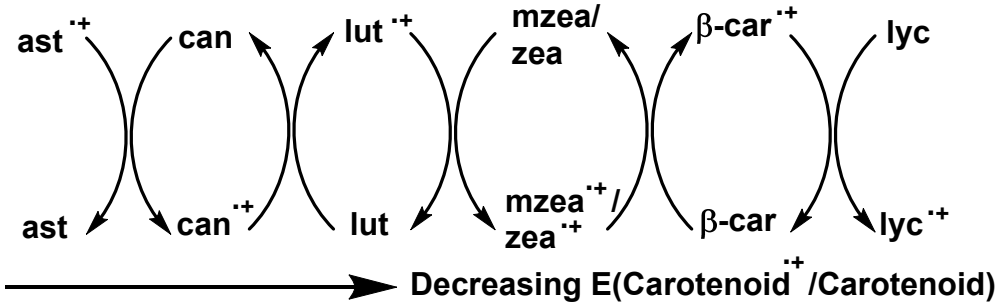
The protonated form of superoxide, the hydroperoxyl radical ($HO_2^{\bullet}$), is, however, much more reactive and can initiate lipid peroxidation, unlike superoxide itself, and produces sequence specific DNA damage [24]. It has a pKa of 4.8 so that, at least at physiological pH, there is only a very small amount of the protonated form present. Both species are able to react with themselves and each other to produce hydrogen peroxide, which can go on to react with more superoxide to yield the much more reactive hydroxyl radical, and this reaction is enhanced in the presence of metal ions, such as iron.

Peroxyl radicals are most often formed via the direct reaction of carbon-centred free radicals with oxygen and are produced in vivo during lipid peroxidation. Radicals such as $OH^{\bullet}$ or $HO_2^{\bullet}$, or even singlet oxygen, react with a polyunsaturated fatty acid (PUFA) residue, producing a PUFA peroxyl radical. This peroxyl radical can react with a second PUFA residue to yield PUFAOOH (a lipid hydroperoxide) and another PUFA carbon-centred radical, thus initiating a chain reaction. However, even if the chain reaction is terminated, the accumulated lipid hydroperoxides are able to react with in vivo metal complexes, producing further radical species.

The most studied peroxyl radical, especially its reactions with a variety of antioxidants, is trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$). In vivo it causes hepatoxicity and is generated during carbon tetrachloride metabolism [25].

A range of antioxidants quench this radical species [26,27], usually via electron transfer, but with carotenoids another reaction also occurs, suggested to be an addition reaction [28]. In Hill et al. it is shown that the electron transfer reaction with the carotenoid ast proceeds only via the suggested addition radical [28]. This implies that the one-electron reduction potential of ast radical cation may be very close to that of $CCl_3O_2^{\bullet}$ and, as such, the electron transfer is very slow.

Indeed, the one-electron reduction potential of ast radical cation is higher than those of many other dietary carotenoids [29]. This is shown in Scheme 1 below, where it can be seen that of the different carotenoids studied, lyc radical cation has the lowest reduction potential and ast radical cation the highest. These experiments, however, were conducted in benzene, due to the hydrophobic nature of most carotenoids.



Scheme 1. Relative order of the carotenoid radical cation one-electron reduction potentials in benzene.

Further pulse radiolysis studies of carotenoids in liposomes (a more biologically relevant environment) enabled estimations of the radical cation reduction potentials via carotenoid reactions with tryptophan radicals. These results show that lyc radical cation has the lowest reduction potential (980 mV versus NHE). However, all the other carotenoids studied showed similar one-electron reduction potentials, in the range 1028–1060 mV versus NHE [30].

Nitrogen monoxide, or nitric oxide ($NO^{\bullet}$), is produced in vivo in activated macrophages and neutrophils, to help kill bacteria [31] and also to act as an intercellular messenger and a vasodilator [32]. Photochemical processes can lead to the generation of $NO^{\bullet}$ but, additionally, exhaust fumes and cigarette smoke also contain $NO^{\bullet}$; however, it reacts rapidly with oxygen to generate nitrogen dioxide ($NO_2^{\bullet}$). Additionally, $NO^{\bullet}$ can also rapidly

react with superoxide, generating the non-radical oxidant peroxynitrite ($OONO^-$) [33]. Nitrogen dioxide, like nitric oxide, is an air pollutant, but it is a much more stable gas in air and, surprisingly, it has been demonstrated that it can induce lipid peroxidation [34]. Additionally, the nitrate radical ($NO_3^\bullet$) is also an air pollutant and a strong oxidising agent which can be formed via reaction of ozone with $NO_2^\bullet$.

$NO_2^\bullet$ and $NO_3^\bullet$ are both more oxidising than $NO^\bullet$, each reacting with a range of antioxidants. $NO_2^\bullet$ most often reacts via one-electron oxidation, as observed for β-car [35], though it can also add across double bonds [34]. We have demonstrated that carotenoids protect lymphocytes from $NO_2^\bullet$-induced membrane damage and that the addition of vitamins E and C increases the protection synergistically [17,36]. Vitamin C has been shown to quench carotenoid radial cations [37] regenerating the parent carotenoid, which could be one explanation for the synergism (and may be related to the extremely surprising cancer results as discussed below, where it is shown that β-car can actually be carcinogenic). The reactions of $NO_3^\bullet$ with antioxidants are more complex and can occur via electron transfer, addition, or even hydrogen abstraction [38–40].

Overall, many of the surprising results with carotenoids may well be related to a switch in behaviour from an antioxidant to a pro-oxidant (or no effect) as a function of oxygen concentration, and this is related to oxygen addition processes producing reactive peroxy radicals. This switch was first reported by Burton and Ingold using a complex solvent possibly not related to the biological situation [41]. A similar 'switch' involving oxygen addition reactions has been reported in ex vivo conditions [23], and is related to the protective role of lut and zea in macular degeneration, discussed below. Another example concerning lung cancer has also been suggested [42], and is also discussed below.

4. Carotenoids in the Skin

There are several contradictory aspects of the benefits or deleterious effects of dietary carotenoids in the skin. These range from the clinical treatment of erythropoetic protoporhyria (EPP) and its use as a sunscreen (inhibitor of sunburn) to possible deleterious effects of skin cancers. The major carotenoid used for skin photoprotection is β-carotene, although several others such as zeaxanthin (zea), lutein (lut), and astaxanthin (ast) [43,44] have been studied. But, the value of such dietary supplementation for protection against sunlight is controversial. The role of ast has been recently reviewed [45]. The uptake of ast may be limited and high concentrations may well be needed for a significant effect [46].

There is much commercial interest in ast as a skin protector and skin-whitener (based on reduced melanogenesis) [47]. As noted by these researchers, dietary ast is mainly the all-E isomer, although the Z isomers are also present in the skin. This important study showed that ast enriched in Z-isomers showed greater UV light-shielding ability, anti-ageing, and skin-whitening activity. However, they also claim that the E isomer was significantly (about 2.5 times) more efficient as a singlet oxygen quencher. Earlier work [48] on β-car in organic solvents and micelles also showed somewhat better singlet oxygen quenching for the all-trans isomer compared to the 9- and 15-cis isomers.

Of course, if the different ast isomers undergo significantly different degrees of aggregation, this would explain such results. However, the ast samples were diluted by chloroform for the assay of the singlet oxygen scavenging activity, so that may well not be the explanation, since solubility in chloroform is high, making aggregation unlikely. A direct measurement of singlet oxygen quenching via a pulsed laser/singlet oxygen emission at 1270 nm would clearly be worthwhile to clarify these important results from Honda and Nishida. As discussed above, other xanthophylls, such as lut and zea, have been shown not to quench singlet oxygen when aggregated using water/alcohol solvent at varying ratios [49], and similar experiments may well be worthwhile for the ast isomers.

In recent studies, Ascova et al. [44] used four in vitro oxidative stress models to study the antioxidant/antipollution effects of all-trans-astaxanthin and crocin compared to other antioxidants (alpha-tocopherol, butylhydroxytoluene, butylhydroxyanisole, gallic acid and Trolox). In general, there was little difference between the two carotenoids, apart

from the skin explant studies which showed ast to be the most efficient anti-oxidant in the skin explant studies. The singlet oxygen results they report are somewhat controversial. Photo-activated methylene blue was used to generate singlet oxygen, and a non-direct procedure based on photometric monitoring the oxidation of p-nitrosodimethylanilin by singlet oxygen in the presence of histidine was used. The authors speculate that their results are linked to the triplet level of ast in comparison to other carotenoids. However, it is important to note that only estimates of triplet levels of any dietary carotenoid have been established. Furthermore, using a non-ambiguous direct detection of singlet oxygen from its luminescence near 1270 nm, it has been shown that singlet oxygen quenching with lycopene, containing no carbonyl groups, is more efficient than ast in organic solvents, although the difference is small and probably of no in vivo relevance [48]. Also, even synthetic carotenoids with 15 and 19 conjugated double bonds (which are likely to have significantly lower triplet energy levels) are only around 45% and 65% more efficient singlet oxygen quenchers than ast (and other dietary carotenoids), respectively. We suggest the singlet oxygen results (alone) of Ascova et al. are not convincing as far as comparing ast with other dietary carotenoids—they are all extremely efficient SO quenchers.

We agree with these authors that there are often exaggerated commercial claims of the antioxidant capability of ast compared to other carotenoids and, while there is clear value of ast for skin protection and it is an effective singlet oxygen quencher, such quenching may or may not be significant for dermal protection.

A major benefit of the clinical use of β-car is the treatment of the extreme photosensitivity associated with the disease of EPP. This disease is caused by an enzymic disfunction leading to an excess of protoporphyrin IX (PP) in the skin. It is relatively rare, occurring in about one in every 100,000. The risk of inheriting EPP is about one in ten, and the risk for men and women is the same.

β-Car has been used for over 30 years to ameliorate this skin photosensitivity arising from excess PP. In the early studies of Mathews-Roth and co-workers [50,51], about 84% of those with EPP benefited from large daily doses of β-car. Currently, the normal dose used is 75–150 mg/day. However, as discussed below, such a high dose may lead to increased risk of lung cancer in certain groups such as heavy smokers (who usually have depleted vitamin C levels, see below). This possible detrimental effect needs consideration before β-car treatment of EPP is used, and molecular mechanisms for this behaviour need to be further studied.

Other porphyrins are associated, via other enzymic dysfunction, with different porphyrias. Thus, when uroporphyrin 1 (UP) builds up in the urine and plasma [52], the result is the disease porphyria cutanea tarda (PCT), causing both photosensitivity and actinic elastosis. PCT treatment with β-car has also been studied, but, unlike EPP, there is no significant benefit against PCT.

β-car is also used to treat other skin diseases, such as solar urticaria, polymorphic light eruptions (PLE), and photoallergic drug reactions, although in some cases the outcome seems variable. Mathews-Roth [50] showed only 33% benefited from β-car against PLE and only 20% for other forms of UVB-induced photosensitivity. However, other researchers [53] claim significant benefits for β-car, with over 50 of the 66 patients studied reporting significantly increased tolerance to sunlight. The detailed molecular mechanisms of PLE/β-car and the UV absorbing chromophores are not established (there is no excess porphyrin associated with these diseases). Possibly, UVB absorbers, including the amino acid moieties tryptophan, tyrosine and cysteine, as well as flavins, etc., may be involved. If so, we may expect a range of ROS radicals as well as singlet oxygen to be formed, and at least some of these could be ameliorated (quenched) by β-car.

So, to summarise the differing outcomes for β-car use in porphyrin-related disease protection: (i) it offers good protection against EPP; (ii) it offers no protection against PCT; (ii) it offers some protection against PLE.

Molecular mechanisms may offer some explanations for such disparate behaviour. Thus, both PP and β-car are soluble in lipid, non-aqueous, environments so the carotenoid

can readily quench the triplet state of PP (which is the generator of singlet oxygen) and any singlet oxygen then formed. However, the UP is water soluble and β-car will not be in the same environment as the singlet oxygen generated from UP light absorption. Thus, a photophysical explanation may be considered based on solubility factors. Both porphyrins generate damaging singlet oxygen via light absorption and triplet energy transfer to oxygen. But, the lifetime of singlet oxygen is likely to be much shorter in water than in lipophilic environments—so quenching, and hence protection, by β-car against damaging singlet oxygen from PP can be much more efficient than quenching of singlet oxygen produced by UP in the aqueous phase. However, this may seem contrary to the photophysical studies described above, which showed in liposomal studies that the singlet oxygen quenching efficiency was independent of the site of singlet oxygen generation. Possibly, the concentration of the β-car is important since this may affect the precise location of the carotenoid in the membrane [54], and more work is worthwhile. An interesting method for a better clinical outcome in general may be the use of water-solubilised carotenoids such as ast [55,56].

As discussed above, the use of β-car to treat the photodamage aspects of porphyric-type diseases requires a large dose of the carotenoid (typically 75–150 mg per day). However, there has been much debate as to the relative benefit of this level (and less) because of the possible increased risk of some cancers for certain sub-groups. The β-car concentration in the lung increases with supplementation, but the levels depend on dosage [57].

The well-known so-called CARET and ATBC trials [58–61] show an increased risk of lung cancer due to high-dose β-car supplementation, whereas other, more recent works [62–64] do not show such detrimental effects. Of course, no light reaches the lungs, but photophysical studies [36,37,42] of model systems lead us to suggest that a possible beneficial role for carotenoid plus vitamin C is worthy of study in future carotenoid trials.

In view of the possible pro-oxidant effect and the need for more work on the mechanisms of β-car in vivo, it is suggested that the use of β-car should be discussed with EPP patients in terms of the risk/benefit ratio.

For skin, without excess porphyrins, the use of carotenoids to offer photoprotection has been studied for both humans and animals. In early work on humans [65], Matthews-Roth et al. reported a small but statistically significant effect of β-car in increasing the minimal erythema dose of solar radiation. However, in work on albino hairless mice, Sayre and Black [66] found the light absorbance due to the β-car was insufficient to give significant photoprotection. More recent work also showed modest benefits for both α- and β-car [67,68], and showed the such protection by β-car was improved by the addition of vitamin E. However, this photoprotection was only evident after 10 weeks of dietary supplementation. In shorter supplement trials, no photoprotection was observed [69].

In a meta-analysis of the protection from sunburn with β-car [70], Köpcke and Krutmann showed β-car supplementation protects against sunburn. The analysis showed that protection required a minimum of 10 weeks of supplementation with an increase of the protective effect with every additional month of supplementation so that dietary supplementation with β-car gives protection against sunburn in a time-dependent manner. The molecular mechanisms and light-absorbing chromophores are not fully established.

Overall, there are many variables in such trials using β-car, with the length of time of the supplement diet before exposure being particularly important.

As noted above, for the role of β-car in the treatment of PLE, there are several possible skin UV absorbers, including the aromatic amino acid moieties tryptophan, tyrosine, and also cysteine, as well as flavins, etc. A range of ROS/RNS may then be formed, and at least some of these could be ameliorated (quenched) by carotenoids, such as β-car. As discussed above, such free radicals can lead to complex reactions, some of which will depend on the oxygen concentration, and can lead to a switch from beneficial antioxidative processes to deleterious pro-oxidation.

As also described above, a further, important, complication arises from the work of Ogilby, who found, using single HeLa cells, no quenching of singlet oxygen by β-car [13].

However, other workers do observe such quenching ex vivo [71], with the efficiency of the energy transfer depending on the environment.

Other carotenoids have also been considered as protectors against solar radiation, especially lyc, with trials ranging from tomato juice and tomato paste to lyc itself [72]. Typically, trials with lut and ast also indicate some skin photoprotection [72,73].

Overall, as with β-car, lyc gives modest photoprotection, with protection factors around 3–4 observed. Of course, commercial sunscreens (organic and inorganic UVA and UVB) used during high exposure have protection factors up to 50. However, as suggested by Stahl and Sies (2007) [74], a carotenoid diet may be useful to protect from erythema and wrinkling and improve skin health in general, and carotenoids may be suitable, in appropriate doses, as life-long protectors.

Finally, the effect of carotenoids with respect to skin cancers is considered. A major issue here is whether carotenoids, and especially β-car, are beneficial, deleterious, or are 'neutral' (having little or no effect), as far as cancers of the skin are concerned. It seems likely that variation in outcomes, mainly studied with β-car, are often linked to the switch from the carotenoid as an antioxidant to the carotenoid as a damaging pro-oxidant.

For the skin, we must distinguish the role of β-car on non-melanoma cancer and melanoma skin cancer. Early studies, around 30 years ago, led to contradictory outcomes with both benefits [75] and no benefits [76,77]. In this work, the possible values of β-car on patient sub-groups—age, gender, smoking history, and skin type, as well as numbers of previous skin cancers and baseline plasma β-car levels—were reported. In these case-controlled studies [78,79], β-car supplementation had no effect on any of the controlled patient subgroups.

Few studies have examined the influence of β-car on the occurrence of melanoma skin cancer. In three case-control studies, no association was found between blood carotenoid levels and the risk of melanoma [78–80]. In one of these studies, the risk of melanoma among men and women in the three highest quartiles of β-car intake actually increased by 40–50%, albeit neither the risk for the fourth quartile nor the test for trend was statistically significant [80]. Overall, it seems that dietary β-car is of little or no benefit to any type of skin cancer, which may be surprising since it does offer some protection against erythema and improves skin health over the long term.

There have been contradictory results reported using animals, especially mice, with carefully controlled β-car containing diets and UV irradiation causing an increased number of skin cancers.

One such trial [81] compared β-car, ast, and lyc. This trial showed significant exacerbation of the UV-induced carcinogenic expression by β-car and ast, but no significant effect with lyc. A suggested explanation was based on the relative redox potentials, with lyc being the most easily oxidised carotenoid and lyc being the most rapidly destroyed by the UV. Of course, other factors, such as a role for Vitamin D, may well be important. Also, for lyc, the low levels that accrue in the epidermis could contribute to its lack of carcinogenic activity. This topic has been thoroughly reviewed recently [3].

5. Eye Protection

Age-related macular degeneration (AMD) is the main cause of blindness in old age in the Western world. In addition, environmental/social factors such as smoking also contribute to this problem [82,83]. Such aspects may well be linked to the effects of ROS and RNS species. Three specific xanthophylls, zea, mzea and lut (termed the macular pigments (MP), accumulate in the macula and are believed to be important protectors against age-related macular degeneration (AMD). Only these MP accumulate in the macular from our diet, even though they are present in the diet in rather low amounts compared to β-car. The reason why only the MP accumulate is not clear, but presumably the terminal OH-group, which may anchor the MP in the membrane to the aqueous surface, is a factor. Nevertheless, the macular lutea has concentrations of MP near 1 mM—the highest concentration of carotenoids in the human body, with approximate concentrations of the

individual MPs of 1:1:1. There are three major properties of the MP linked to AMD. A "passive" light absorption inner filter effect and "active" processes associated with their antioxidant properties. It has been estimated that the MP can attenuate up to about 40% of the potentially damaging light [84]. In this review we are mainly concerned with the active antioxidative mechanisms.

A major role postulated for all three MPs is the quenching of singlet oxygen. This is an efficient process in normal organic solvents but shows that zea and mzea are twice as efficient quenchers as lut. So, based on this alone, the need for lut in the macula is not clear. Several possibilities arise. Firstly, we consider simple passive light absorption, since the absorption spectrum of lut is blue-shifted compared to those of zea and mzea. So, the protection against actinic insult covers a wider wavelength range due to the presence of lut. This "passive" role is of importance but is not discussed further in this review.

Two "active" mechanisms, showing the possible value of lut, are of interest. Firstly, carotenoid pigments tend to aggregate in liposomal/membrane environments. Overall, this can be a complex process and is well-reviewed by [12]. In simple terms, aggregated carotenoids quench singlet oxygen with very much lower efficiencies than monomeric carotenoids. Comparisons between lut and zea, using in vitro systems, show more efficient quenching of singlet oxygen by lut, and, in the model systems studied, the aggregation is significantly less for lut than for zea.

The second 'active' role of lut concerns its radical reactions. As discussed above, oxygen concentration can be particularly important with respect to free radical processes due to oxygen addition to neutral free radicals such as $OH^{\bullet}$-adducts. This can produce extremely damaging peroxyl radicals $ROO^{\bullet}$. A typical mechanism could be:

$$\text{lut} + OH^{\bullet} \rightarrow \text{lut-}OH^{\bullet} \quad (1)$$

$$\text{lutOH}^{\bullet} + O_2 \rightleftharpoons \text{lutOH-}OO^{\bullet} \quad (2)$$

$$\text{lutOH-}OO^{\bullet} + \text{Cells} \rightarrow \text{Cell Damage} \quad (3)$$

We have discussed cell damage linked to several carotenoids based on this mechanism, including lut and zea [23]. Overall, we used gamma radiation to generate specifically $OH^{\bullet}$ and $O_2^{\bullet -}$, and compared the cell kill by lutOH-$OO^{\bullet}$ and zeaOH-$OO^{\bullet}$ as a function of oxygen concentration from zero to 100%. We obtained protection factors with values of about 26 for lut and 8.7 for zea in air (at higher oxygen concentrations, the ratio is even more dramatic, with the corresponding protection factors of 25 and 3.3). We propose the equilibrium position for equation 2, and the corresponding equation for zea is such that it lies further to the left for lut than zea, so that there is much less lutOH-$OO^{\bullet}$ formed than zeaOH-$OO^{\bullet}$. So, overall, lut may have three significant photochemical-related benefits for macular protection.

Experiments were also reported in which a mixture of $OH^{\bullet}$ and $O_2^{\bullet -}$ were generated in approximately equal amounts. Of course, cells are protected against $O_2^{\bullet -}$ damage by SOD, and we found such SOD protection was independent of the oxygen concentration.

Comparing the protective effects of SOD plus zea and SOD plus lut showed the lut-containing system to be about three times as effective at 20% oxygen compared to the zea-containing system. So, this may offer an additional protective role for lut.

In very recent work on ast [85], also using gamma radiation, it has been reported that for normal (BHK-21 and CHOK1) cells, ast improves cell viability, reduces the intracellular ROS levels and DNA damage, and suppresses radiation-induced apoptosis. Also, ast mediated the cellular protection effect by activating the Nrf2 signalling pathway and upregulating the expressions of Nrf2, p-Nrf2, HO-1, and other antioxidant proteins.

As noted earlier, carotenoids can switch from being efficient antioxidants to non-protective and even to pro-oxidants, depending on the oxygen concentration, and this has also been shown to be so for zea and, to some extent, for ast protection of lymphocyte

cells [23]. It would be of interest to extend the important results of Zheng and co-workers to conditions, such as high oxygen concentrations, where other xanthophylls show much-reduced or no cell protection.

Another aspect of eye protection concerns the roles of vitamins E (consisting of tocopherols and tocotrienols) and C. Tocopherols are well known as chain-breaking antioxidants, and vitamin C is also an effective antioxidant beneficial to the eye. This review [86] concerns carotenoids and xanthophylls and their interactions with vitamin C, possibly leading to unexpected synergistic antioxidant properties, which may well be important.

Also, Rózanowska and coworkers [87], using esr and a xenon light source at 404 nm, have shown that in combination with vitamin E, ascorbate can offer significant protection of RPE cells from such irradiation. These results showed that single lipophilic antioxidants had only a minor effect on phototoxicity, but the protection substantially increased in the presence of antioxidant combinations. For example, zea plus α-tocopherol enhanced cell viability, and this protective effect was further increased in the presence of ascorbate. However, the protective effect of ascorbate disappeared at a concentration of 1 mM, whereas 2 mM of ascorbate exacerbated the phototoxicity. Zea or α-tocopherol partly ameliorated the cytotoxic effects. Also, the products of the oxidative degradation of the xanthophylls have been shown to cause the generation of reactive oxygen species and apoptosis [88]. Two of these products, namely 3-hydroxy-β-ionone and 3-hydroxy-apocarotenal, are both likely derived from oxidative cleavage of lut or zea, and have been identified in human retinas postmortem [89]. Therefore, increasing, by supplementation, the concentrations of lipophilic antioxidants, such as vitamin E, lut, or zea, must be used with caution.

A final, interesting, controversial, and much-debated aspect of MP protection is the role of dietary lyc. Of course, there is no lyc in the macula. Nevertheless, there are substantial claims [90] that high-lyc diets can be beneficial in the protection of the macula—how can this be so? A photochemical molecular mechanism is a possibility, as explained below.

The reactivity of carotenoids as antioxidants depends on their redox potentials, and these have been determined in aqueous micellar solutions for various dietary carotenoids [29,30], and the relative one-electron reduction potentials are shown in the figure above.

As can be seen, lyc has the lowest redox potential, i.e., it is the most easily oxidised of the dietary carotenoids studied. Of course, there is a mixture of many carotenoids in the typical diet, so that the quenching of any free radical could occur by any of the carotenoids. But, if such quenching is by lut, zea, or mzea (yielding the corresponding radical cations via electron transfer), there is at least the possibility that a further electron transfer, as in the above figure, will occur, regenerating the 'parent' MP and producing $lyc^{\bullet+}$. Thus, lyc acts as the 'sacrificial' carotenoid antioxidant, reducing any loss of MP and thus allowing a greater amount of MP to reach the eye.

6. Concluding Remarks

Dietary carotenoids have a wide range of roles, from food colourants to photosynthesis to human health. Furthermore, they have 'flexible' properties, offering benefits but also possible deleterious health effects. In photochemical studies, they can switch from being extremely efficient antioxidants to potentially damaging pro-oxidants, and this may well provide some molecular mechanisms for the varying and often apparent contradictory properties of the dietary carotenoids. A wide range of studies, from fundamental photophysical to in vivo and epidemiological, are still needed to unlock the full potential of dietary carotenoids in human health.

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