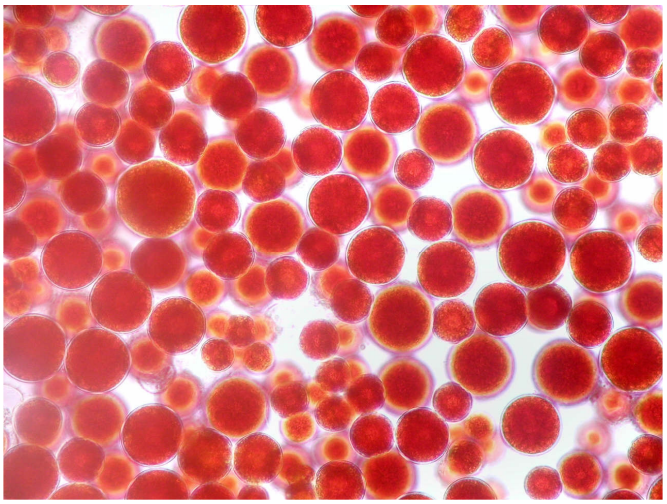
Astaxanthin and Human Health

Literature Survey



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(1) Astaxanthin as a powerful antioxidant

As aerobic organisms, we depend completely on molecular oxygen for our existence; the typical result of just a few minutes without oxygen is irreparable damage or death. However, although oxygen is utterly critical for human life, this molecule has also a dark side to its actions. Oxygen is also found in a large number of harmful by-products that are relentlessly being produced in living tissues. These molecules are chemically unbalanced and very active; hence they tend to react with any other adjacent molecule. These *reactive-oxygen species* (ROS) contain reduced oxygen molecules as free radicals and reactive compounds.

In nature, electrons in covalent bonds always come in pairs. Whenever a covalent bond is broken down, each atom is left with one unpaired very active electron, and is therefore termed a *free radical*. Free radicals include superoxide, hydroxyl radicals, and peroxyl radicals; all have one unpaired electron, and thus will seek any other atom with which to react.
ROS also include reactive compounds, which are non-radicals, such as ozone, lipid peroxides, hydrogen peroxide, and singlet oxygen. Additionally, a number of nitrogen compounds containing oxygen, such as nitrogen oxides and peroxynitrite, are also extremely harmful.

The strong tendency of ROS to react with neighboring molecules puts these molecules at risk. Note that free radicals and highly reactive forms of oxygen are produced in the human body during normal metabolic reactions and processes. Consequently, ROS are found in our bodies at any given time, and react with the tissue molecular constituents, such as proteins, DNA, RNA, carbohydrates, and lipids. The results of such “oxidative attack” may include protein and lipid peroxidation and structural changes in DNA and RNA, which in turn may lead to damage, mutations, and even loss of function. The oxidation of poly-unsaturated fatty acids in the membranes could induce a chain reaction of free radicals, which in turn could result in the loss of adequate function of the lipid components of the cellular membranes.

Physiological stress, air pollution, tobacco smoke, exposure to toxic chemicals, or exposure to ultraviolet (UV) light can enhance the production of ROS. Indeed, oxidative damage has been linked to aging, atherosclerosis, ischemia-reperfusion injury, macular degeneration of the eye, carcinogenesis, neurodegenerative diseases, bacterial and viral meningitis, and many other known health phenomena and diseases, all of which pathogenic conditions involve an underlying oxidative insult, either in their development or in their progression.

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On the other hand, this constant attack on the body is continuously countered by mechanisms designed to neutralize oxidative damage and prevent associated damage and diseases. An important defense mechanism in the body is the cascade of enzymes that neutralize the ROS prior to the induced damage (superoxide dismutase, catalase, glutathione peroxidase). This preventive pathway is extremely important, since it helps to support a healthy existence. Certain repair enzymes can reverse the damage produced by the ROS, as in the case of DNA breaks being enzymatically restored.

An additional defense mechanism against free radicals and reactive compounds in the body requires the action of special molecules, ones we call *antioxidants*. Antioxidants are a variety of substances from diverse chemical groups that share one common property: their ability to scavenge for the harmful free radicals and react with these active molecules. Some of the antioxidants in our defense system are synthesized in the body; some are solely consumed with the diet. It is no wonder, therefore, that there has been much interest in investigating the use of such compounds to slow the progression of, and in some cases even prevent, a wide array of health phenomena and diseases.

Of the antioxidants investigated for their potential health benefits, the *carotenoids* have rightfully received wide attention. Carotenoids are lipid-soluble pigments extracted from plants, algae, and some fungi and bacterial species that account for the remarkable red, orange, or yellow hues observed in many carotenoid-rich organisms. In many underwater and land species, the flamboyant body coloration results from mixing some of these over 700 known carotenoids.

In the plant kingdom, algae and phytoplankton carotenoids also participate in photosynthesis, by acting as secondary, light-absorbing molecules in the photosynthetic antenna. Carotenoids are antioxidants due to their ability to quench singlet oxygen, be oxidized, and isomerized (Mortensen et al., 1997). All in all, even carotenoids' pigmentation property is connected to their antioxidant chemical nature: They absorb light due to their special bonding structure, and hence provide defense from excessive light radiation and photo-oxidative damage. This property can also explain carotenoids' prevalence in plants and algae, which, unlike animals, lack motion capabilities, and hence cannot escape continuous exposure to light. Animals are unable to endogenously synthesize carotenoids, although some can alter carotenoids into other forms. Nevertheless, animals do accumulate carotenoids through their diet, as will be discussed herein.

The carbon backbone of a typical dietary carotenoid consists of a long chain that might contain hexagon rings at both ends. While the chain itself is

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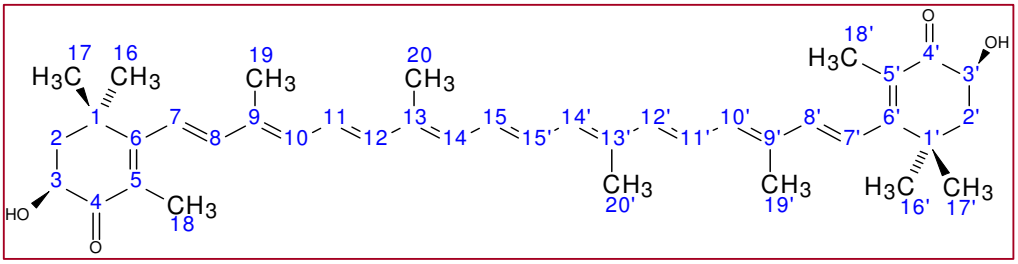
uniform, the terminal rings may bear various chemically active groups, each determining unique chemical characteristics. Oxygen-free carotenoids are called *carotenes*, while oxygen-containing carotenoids are called *xanthophylls*. The special structures of carotenoids allow them to react with ROS, thereby absorbing the latter's excess energy, and releasing it as heat; in certain cases, this heat-releasing process leads to the degradation of the carotenoid molecule, yet prevents other molecules or tissues being damaged (Mortensen et al., 2001; Rousseau et al., 1992). However, not all carotenoids are equal, and the differences in their chemical structure account for the specific activities of the individual carotenoid.

Astaxanthin is a carotenoid that belongs to the xanthophyll sub-group, a family of oxygen-containing carotenoids. Unlike the most common carotenoid in the human diet, the Vitamin A precursor β-carotene, Astaxanthin possesses additional potent hydroxyl and ketone groups at both termini, which are responsible for its official chemical name, 3,3'-dihydroxy-β-β-carotene-4,4'-dione. Astaxanthin has two asymmetric carbons (carbons 3 and 3') in its side rings, and thus contains two chiral centers. Therefore, it may present three stereoisomers: 3S, 3'S form, 3R, 3'R form, and the meso form 3R, 3'S. Synthetic Astaxanthin consists of the racemic mixture of the three enantiomers, but only one form is abundant naturally: the 3S, 3'S isomer.

Astaxanthin consists of geometric isomers as well, all-trans isomer (all-E), and the cis isomers (mainly as 9Z and 13Z). In nature, Astaxanthin can appear as free Astaxanthin, monoester, or diester; while the most abundant geometric isomer in nature is the all-E isomer. In the microalgae *Haematococcus pluvialis*, Astaxanthin is accumulated mainly as monoester, partly as diester, and only in minor quantities as free Astaxanthin.



Chemical structure of Astaxanthin.

The biosynthesis of Astaxanthin proceeds through a number of important intermediates including phytoene, lycopene, β-carotene, zeaxanthin, and canthaxanthin. Ingestion of Astaxanthin in the food is reflected in many

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aquatic animals, explaining the rich pink color observed in salmon and crustaceans such as crabs, lobster, and shrimp; even the pink pigmentation of the flamingo accounts for its Astaxanthin-rich diet (Maher, 2000). The richest source of Astaxanthin known today by far is the algae *Haematococcus pluvialis*, which can accumulate more than 40 gr of Astaxanthin per kilogram of dry biomass of the 3S, 3'S enantiomer.

Experimentally, the potency of an antioxidant to chemically neutralize and scavenge harmful oxygen reactive compounds can be determined by using *in vitro* systems in the laboratory. One such assay measures the production of ROS-induced lipid peroxides in test tubes both in the absence and in the presence of a tested antioxidant. As early as 1989, researchers showed that Astaxanthin and canthaxanthin retard hydro-peroxide formation more efficiently than β-carotene and zeaxanthin (Terao, 1989). The rate of autocatalytic oxidation of Astaxanthin was also slower, suggesting that its effectiveness as an antioxidant is by stabilizing the trapped radicals. Miki (1991) has shown that Astaxanthin had the strongest quenching effect against singlet oxygen, and a strong scavenging effect against free radicals. Astaxanthin was found to be at least 10 times stronger antioxidant than zeaxanthin, lutein, tunaxanthin, canthaxanthin, and beta-carotene, and 100 times stronger than Vitamin E.

Astaxanthin also showed strong activity as an inhibitor of lipid peroxidation mediated by active forms of oxygen. Astaxanthin concentrations of 200 nM were sufficient to cause 50% inhibition (ED_{50}) of lipid peroxidation, while 400 nM of zeaxanthin, 700 nM of lutein, 960 nM of beta-carotene, and 2940 nM of Vitamin E were required to cause the same inhibition of lipid peroxidation. Following these findings, Simidzu and coworkers were looking at the quenching activity (*in vitro*) of singlet oxygen of eight major carotenoids prevalent in marine organisms (Shimidzu et al. 1996). Astaxanthin was found to be the most active carotenoid in quenching singlet oxygen, more then zeaxanthin, lutein, beta-carotene, and canthaxanthin. The researchers suggest that these carotenoids may play a major role in protecting marine organisms from active oxygen species.

These intriguing observations were later confirmed in an improved experimental system, using liposomes (Nishigaki et al., 1994; Palozza and Krinsky, 1992). When the conjugated keto-carotenoids, either Astaxanthin or canthaxanthin, were added to rat liver microsomes undergoing radical-initiated lipid peroxidation, they were as effective as alpha-tocopherol in inhibiting this process. This result contrasted with the effect of beta-carotene, which was found to be a less potent antioxidant when added into this system without the addition of other antioxidants.

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Striving toward a better model, scientists looked for a system that mimics natural occurring conditions. In this aspect, initiation of the oxidative damage was not chemically induced, but rather the outcome of light radiation (Oshima et al., 1993). Large liposomes comprised of egg yolk phosphatidylcholine (PC) were exposed to photo-irradiation in the presence of a photosensitizer to estimate the inhibitory effect of β-carotene and Astaxanthin on the oxidation of phospholipid bilayers. Without sensitizers, Astaxanthin decreased much more slowly than did β-carotene and other carotenoids, including lycopene and alpha-carotene. Astaxanthin lasted longer than β-carotene even in the presence of the photosensitizer. These results suggest that the improved stability of Astaxanthin on photoirradiation increases its ability to act as an effective antioxidant in this system.

Iron ions are common compounds in biological cellular environments, and are known for their pro-oxidative characteristics. Therefore, the addition of iron to oxidation reactions was the next step in evaluating the antioxidant effects of Astaxanthin (Nakagawa et al., 1997). Rat liver microsomes were exposed to a mixture of chelated iron (Fe3+/ADP) and NADPH. Astaxanthin was incorporated into some of these microsomal membranes, and both phospholipid hydro-peroxides (PLOOH) and endogenous alpha-tocopherol content were measured over time after the initiation of oxidant stress. In control microsomes, oxidant stress led to the accumulation of 1,865 pmol PLOOH / mg protein during the initial 10-minute peroxidation reaction, followed by a more gradual decrease during the subsequent 20 minutes of reaction. With Astaxanthin present, PLOOH accumulation during the initial 10-minute reaction period was reduced to 800 pmol / mg protein. During the following 20 minutes of incubation, PLOOH levels declined in the carotenoid-supplemented microsomes, yet continued to increase (albeit at a slower rate) in control preparations. The presence of Astaxanthin in the microsomal membrane partially inhibited the loss of alpha-tocopherol, especially during the later phase of oxidant stress. A similar antioxidant effect of Astaxanthin was shown in the presence of copper ions in another independent study (Rengel et al., 2000).

Research procedures evolved, and the antioxidant activities of Astaxanthin and related carotenoids have been measured by employing a newly developed fluorometric assay (Naguib, 2000). In this assay, three categories of carotenoids were examined, namely, the hydrocarbon carotenoids lycopene, alpha-carotene, and β-carotene; the hydroxy carotenoid lutein; and the alpha-hydroxy-ketocarotenoid Astaxanthin. In liposomal suspension, Astaxanthin showed the highest antioxidant activity toward peroxyl radicals: the relative reactivities of Astaxanthin, alpha-tocopherol, alpha-carotene, lutein, β-carotene, and lycopene were determined to be 1.3, 0.9, 0.5, 0.4, 0.2, and 0.4, respectively.

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Insights into this powerful protection mechanism emerged in recent studies (Barros et al., 2001; Goto et al., 2001). The first study proposed the hypothesis that the conjugated chain moiety and terminal ring moieties of the more potent Astaxanthin trapped radicals both on the membrane surface and in the membrane, whereas the conjugated chain of β-carotene was responsible for radical trapping merely near the membrane surface and in the interior of the membrane. In order to test whether the antioxidant activity of carotenoids was also related to their effect on membrane permeability, the second study presented an appropriate model. It was shown that the antioxidant activity of Astaxanthin in iron-containing vesicles might be derived from its inherent scavenging ability, as well as its known rigidifying effect, limiting the penetration of lipid peroxidation promoters. In conclusion, Astaxanthin has been shown to be up to 10 times more potent than other carotenoids against a wide array of ROS; its combination of superior potency and versatility yield the ideal antioxidant.

How relevant are these results to human biological systems? As coming chapters will bring ample evidence of Astaxanthin's antioxidant activities in multiple physiological systems and conditions, several studies show biological antioxidant activity *per se*. A quite interesting study in grass shrimp embryos indicates that the natural role of Astaxanthin can never be overestimated (Winston et al., 2004). During embryogenesis in grass shrimp, the capacity to scavenge oxy-radicals increased as measured by the total oxy-radical scavenging capacity assay. This increase was associated with increasing concentrations of a number of antioxidants, including Astaxanthin and β-carotene, which were identified in embryos, with Astaxanthin always the principal carotenoid.

In early embryo stages, there are maternally derived antioxidants, but as embryogenesis proceeds, there is an assembly of a complex antioxidant system by newly formed cells and tissues. A previous study (Lawlor and O'Brien, 1995) showed that these effects are not restricted to embryos of lower organisms, where Astaxanthin is naturally synthesized, but also appear in avian embryos. Lawlor and coworkers evaluated the antioxidant activity of Astaxanthin against paraquat-induced oxidative stress in primary cultures of chicken embryo fibroblasts (CEF). In fibroblasts exposed to oxidative stress, the activities of the antioxidant enzymes superoxide dismutase and catalase significantly increased, while the activity of glutathione peroxidase decreased. Incorporation of Astaxanthin (0.1-10 nM) into the paraquat-treated fibroblast medium reduced SOD and catalase activities, and brought glutathione peroxidase activity to its control value.

Moreover, Astaxanthin enrichment of the growth medium of the paraquat-stressed CEF returned all antioxidant enzyme activities to those seen

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in control cells. A few years later, the same group compared the protective effect of Astaxanthin, lutein, and beta-carotene on the antioxidant enzymes and lipid peroxidation in cultured rat kidney fibroblasts irradiated with UV light (O'Connor and O'Brien, 1998). UV radiation caused significant decrease in the activity of SOD and catalase, and increase in lipid peroxidation. Enrichment of the media with beta-carotene (1 µM), lutein (1 µM), and Astaxanthin (10 nM!) during UV radiation protected the cells against UV light-induced oxidative stress, with Astaxanthin exhibiting superior protective properties (effectiveness at much lower concentrations).

Measuring antioxidant activity *in vivo* was never trivial, yet two independent studies in rats broadened the antioxidant effects of Astaxanthin to living adult mammals (Nishigaki et al., 1994; Kurashige et al., 1990). The first group showed that Astaxanthin protects the mitochondria of Vitamin E-deficient rats from damage by Fe_2(+)-catalyzed lipid peroxidation, both *in vivo* and *in vitro*. This inhibitory effect of Astaxanthin on mitochondrial lipid peroxidation was stronger than that of alpha-tocopherol. In addition, thin layer chromatographic analysis showed that the change in phospholipid components of erythrocytes from Vitamin E-deficient rats under oxidative stress was significantly suppressed by Astaxanthin. The second group not only confirmed these results, but also showed similar effects after induction of oxidative stress by Co-60-irradiation.

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(2) Astaxanthin: Bioavailability and pharmacokinetics

Our diet is among the primary determinants of the antioxidant status in our body, and the metabolic transformation and body load of carotenoids are heavily affected by the quantities consumed. Absorption of carotenoids from the diet occurs by passive diffusion into the intestinal epithelium, a process that requires and is accelerated by small quantities of lipids, and is facilitated by pancreatic phospholipase A2 and lysophosphatidylcholine (Van Het Hof et al., 2000). Following absorption, carotenoids are incorporated into lipoproteins, transported to the liver via lymph and blood, and partly re-secreted with lipoproteins.

Structural differences including geometrical E/Z isomerization cause individual patterns of absorption, plasma transport, and metabolism for each carotenoid. The polar xanthophylls (oxygen-containing carotenoids) and the non-polar carotenes are usually distributed differently among the lipoprotein fractions, the carotenes mainly being present in low-density lipoproteins (LDL), whereas xanthophylls are more equally distributed between LDL and high density lipoproteins (HDL) (Coral-Hinostroza et al., 2004). Astaxanthin has two chiral centers, one in each ring, and consists of three discrete optical R/S isomers; these configurations might influence Astaxanthin's overall absorption and distribution as well.

The first thorough bioavailability study of Astaxanthin in humans was reported at the turn of the millennium (Osterlie et al., 2000). Appearance, pharmacokinetics, and distribution of Astaxanthin E/Z and R/S isomers in plasma and lipoprotein fractions were studied in three middle-aged male volunteers (37-43 years) after ingestion of a single meal containing a 100 mg dose of synthetic Astaxanthin. The Astaxanthin source consists of 74% all-E, 9% 9Z, 17% 13Z, with a ratio of 1:2:1 between the 3R, 3'R form, 3R, 3'S form, and 3S, 3'S stereoisomers. The plasma Astaxanthin concentration-time curves were measured over 72 hours. Maximum levels of Astaxanthin (1.3 mg/L) were reached 6.7 hrs after administration, and the plasma Astaxanthin elimination half-life was 21 hrs. 13Z-Astaxanthin was selectively accumulated in the plasma, but no selectivity was found for any of the stereoisomers. Astaxanthin was present mainly in very low-density lipoproteins containing chylomicrons (VLDL/CM; 36-64% of total Astaxanthin), whereas low-density lipoprotein (LDL) and high-density lipoprotein (HDL) contained 29% and 24% of total Astaxanthin, respectively.

An additional study was published four years later, examining the pharmacokinetics of administrated fatty acyl diester Astaxanthin consisting of a mixture of geometrical and optical isomers (Coral-Hinostroza et al., 2004). Three middle-aged male volunteers (41-50 years) ingested a single meal

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containing first a 10-mg dose equivalent of Astaxanthin diester, followed by a dose of 100 mg Astaxanthin equivalents four weeks later. The plasma Astaxanthin concentration-time curves were measured during 76 hours. Astaxanthin esters were not detected in plasma, indicating that they are hydrolyzed selectively before or during absorption. Maximum levels of Astaxanthin (0.28 mg/l) were reached 11.5 h after administration, and the plasma Astaxanthin elimination half-life was 52 h. At the low dose, the maximum blood concentration was 0.08 mg/l, showing that the dose response was non-linear. The Astaxanthin Z-isomers were absorbed selectively into the plasma compared to the all-E-Astaxanthin; some selectivity was shown for the 3R, 3'R as well. Moreover, the distribution of the geometrical isomers among the various lipoproteins showed selective process, where the main part of the plasma Astaxanthin was present in the VLDL/CM fraction.

It is known that the bioavailability of carotenoids, which are strongly lipophilic compounds, is low, varying widely from less than 10% in raw uncooked vegetables to more than 50% in oily solutions or in synthetic commercial formulations. Odeberg and coworkers were studying the effect of various lipid-based formulations on the bioavailability of algal Astaxanthin in humans. 32 healthy volunteers received a single dose of 40 mg algal Astaxanthin as various lipid-base formulations. The elimination half-life of the Astaxanthin in the plasma was 15.9 hours. All lipid-base formulations showed enhanced bioavailability ranging from 1.7 to 3.7 times that of the reference formulation (which was 55 µg/l). The highest bioavailability was observed with a formulation containing the hydrophilic surfactant polysorbate 80. This research demonstrated that Astaxanthin bioavailability, as for other carotenoids, can be increased by consumption of Astaxanthin together with oils and fats.

In order to learn more about the distribution of digested Astaxanthin, researchers turned also to animal models (Showalter et al., 2004). Oral bioavailability of natural and synthetic carotenoids is generally poor in rodents, and hence Astaxanthin derivatives were designed to increase the percentage of the total oral dose absorbed by the rodent gastrointestinal tract. In the current study, a disodium disuccinate diester of Astaxanthin was orally administered in a lipophilic emulsion to mice. Plasma appearance and tissue accumulation of non-esterified, free Astaxanthin was studied by HPLC over 72 hours after single- and multiple-dose regimens. One-time dosing of Astaxanthin in emulsion at 500 mg/kg resulted in significant appearance of free Astaxanthin in plasma (0.2 mg/l; 381 nM), with higher accumulation in solid organs, such as the liver (0.9 mg/l; 1735 nM). At each point in the concentration / time curve, free Astaxanthin levels in the liver were greater than the corresponding concentration in plasma, suggesting concentrative uptake by the liver. Apart from this hepatic accumulation, this study stresses

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the advantage of using the disodium disuccinate diester form of Astaxanthin (having improved water solubility). This approach is expressed in another study, wherein Astaxanthin was delivered to human colon cells with the emulsifiers Tween 40 and Tween 80 (O'Sullivan et al., 2004).

Having said above that the bioavailability of carotenoids is affected by dietary fat content, additional research tested the influence of various fats (Clark et al., 2000). The effect of oils on the absorption of carotenoids was investigated in mesenteric lymph duct-cannulated rats. Sixteen treatment emulsions containing increasing concentrations of either lycopene or Astaxanthin (5, 10, 15, 20 μmol / L) were prepared with olive oil or corn oil and continuously infused into the duodenum of the rat. Absorption of carotenoids into the mesenteric lymph duct was determined, and as expected, seemed to increase with the amount infused into the duodenum. The average recovery of Astaxanthin in the lymph from the olive oil emulsion was 20%, yet decreased to 13% from emulsions containing corn oil. Lycopene was not as well absorbed as Astaxanthin: Its average recovery of was 6% from olive oil emulsions and only 2.5% when infused with corn oil. One reason for the lower absorption rate in corn oil is that oils high in polyunsaturated fatty acids (PUFA) might promote carotenoid oxidation in the intestine, resulting in less carotenoid available. Another explanation may be that the transfer of the carotenoid from lipid emulsions containing large amount of PUFA to mixed bile salt miscelles is reduced.

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(3) Astaxanthin and eye health

A primary source for oxidative stress is incoming sunlight, which contains ultra violet (UV) light. Blue and purple light is highly energetic as well, and can produce high levels of harmful reactive compounds and various free radicals. Any tissue exposed to light is prone to undergo this photo-oxidative damage, and hence our skin and eyes are the most vulnerable tissues. It is not surprising, therefore, that carotenoids play an essential role in the maintenance of human skin and eyes, protecting them from the adverse effects of UV light. Add to this, the high concentration of polyunsaturated fatty acids in the center of the retina photoreceptive membranes renders the eye a perfect target for lipid peroxidation.

Focusing on the eye, carotenoids have even greater importance, since the visual process of light absorption to produce images is enabled by the action of Vitamin A, a metabolic byproduct of β-carotene. Any compound functioning in the eye must traverse the blood-retinal barrier, which is similar in both structure and function to the blood-brain barrier, about which we know much more. This specialized structure, which helps to prevent the unchecked passage of agents into the central nervous system (CNS) from the periphery, regulates which substances will pass (Maher, 2000).

Among all antioxidants and specifically carotenoids, Astaxanthin appears to easily penetrate the CNS, thanks to its low molecular weight (<600 Dalton), and its specific lipophilic character (hydrophilic side groups attached to the lipophilic skeleton). Indeed, a very high density of Astaxanthin was found in the eyes of some avian species, specifically marine birds, which encounter in their habitats the harmful effects of glare off the water, and need enhanced visual acuity due to the air/water interface as well. In mammals, Astaxanthin will deposit in the eye, similarly to lutein (Maher, 2000; Tso and Lam, 1996).

Although Astaxanthin has not been isolated in the human eye, lutein and zeaxanthin, two carotenoid pigments closely related to it, are concentrated in the center of the retina, or the *macula* of the eye. Two of the main causes of visual impairment and blindness are Age-Related Macular Degeneration (AMD) and Age-Related Nuclear Cataracts (Guerin et al., 2003). Both diseases appear to be related to light-induced oxidative processes within the eye, and hence oxidation factors are positively correlated with elevated risk for these diseases. Moreover, a reduced risk for AMD and nuclear cataracts is associated with a high dietary intake of carotenoids from leafy green vegetables.

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The protective effects of Astaxanthin itself were demonstrated in several animal experiments. Following intraperitoneal administration of Astaxanthin to rats, an Astaxanthin content of 0.17µg / mg was measured in the rats' retinas, thus showing that Astaxanthin can cross the Blood-Retina Barrier (Tso and Lam, 1996). Following Astaxanthin administration, the rats were exposed to visible light for 24 hours. While rats treated with vehicle lost ~35% of the thickness of the outer nuclear layer of the retina, those treated with Astaxanthin had only a 6 % decrease. In addition, Astaxanthin was able to prevent the depletion of rhodopsin levels in the retinas of rats treated in similar photo-damage conditions. In Atlantic salmon (Salmo salar L.), cataract was shown to be significantly reduced by high dietary levels of Vitamin C and Astaxanthin (Waagbo et al., 2003). These studies suggest that Astaxanthin can reduce and prevent conditions of visual disorder.

The effects of Astaxanthin on endotoxin-induced uveitis (EIU, or degeneration of the pigmented vascular coat of the eye) was studied in rats (Ohgami et al 2003). The uveitis was induced by injection of polysaccharides, and Astaxanthin was administrated intravenously. The results indicated that Astaxanthin had a dose-dependent anti-inflammatory effect on EIU, having a possible mechanism of suppressing the production of nitric oxide (NO). This study will be discussed in more details latter on.

Additional work looking at the effect of Astaxanthin on EIU and eye health was done in collaboration between Japanese scientists from Hokkaido University of Medicine and the Tokyo Graduate School of Fisheries Science (Suzuki et al., 2005). Over the course of the eye disease, the researchers measured the expression of inflammatory cytokines and chemokines in the presence or absence of Astaxanthin (1, 10, or 100 mg/kg), which was injected intravenously immediately after the inoculation. Rats injected with Astaxanthin showed a significant decrease in the number of infiltrating cells in the anterior chamber, and additionally, there was a significantly lower concentration of protein, NO, TNF-alpha, and PGE2 in the aqueous humour. Moreover, even early stages of EIU were suppressed by injection of Astaxanthin. These results suggest for the first time that Astaxanthin reduces ocular inflammation in eyes infected with EIU by down-regulating pro-inflammatory factors and by inhibiting the NF-kappaB-dependent signaling pathway. Moreover, it shows that Astaxanthin not only protects the eyes, but also blocks the biologic pathway leading to inflammation.

The inverse relationship between the intake of Vitamins A, C, E, several carotenoids, and the development of certain types of AMD has already been shown in works published in the late 1990s. Yet, it was only in 2004 that Astaxanthin itself was directly shown to be a potent contributor to human eye health (Chitchumroonchokchai et al., 2004). This American group examined

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the effects of xanthophylls and alpha-tocopherol on lipid peroxidation and the mitogen-activated stress signaling pathways in human lens epithelial (HLE) cells following ultraviolet B light (UVB) irradiation. When pre-incubated with lutein, zeaxanthin, Astaxanthin, and alpha-tocopherol (alpha-TC), HLE cells accumulated the antioxidants in a concentration- and time-dependent manner. Pretreatment of cultures with either 2 µmol / L xanthophyll or 10 µmol / L alpha-TC for four hours before exposure to UVB radiation decreased lipid peroxidation by 47-57% compared with UVB-treated control HLE cells. Pretreatment with the xanthophylls and alpha-TC also inhibited UVB-induced activation of stress signaling molecules by 50-60% and 25-32%, respectively. These data suggest that the xanthophylls lutein, zeaxanthin, and Astaxanthin act to decrease UVB-induced lipid peroxidation and attenuate activation of the stress signaling pathways in HLE cells, and are more potent than alpha-TC in protecting human lens epithelial cells against UVB insult.

Can Astaxanthin improve human eyestrain and visual function? Nagaki et al (2002) looked at the effect of Astaxanthin on the degree of eyestrain in visual display terminal (VDT) workers. The visual function of VDT workers that took 5 mg / day of Astaxanthin for four weeks was compared to that of VDT workers that took only placebo for the same period. The results indicated that accommodation amplitude (diopters) after Astaxanthin treatment was significantly higher than before supplementation, while for the placebo group it was unchanged, demonstrating improved specific visual function for VDT workers after Astaxanthin supplementation. An additional study examined the effect of Astaxanthin on visual function of healthy human volunteers over 40 years of age (Nakamura et al 2004). Following ingestion of Astaxanthin for 28 days, the uncorrected far vision acuity significantly improved in groups receiving 4 mg and 12 mg per day; accommodation time was significantly shortened in these two groups as well.

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(4) Astaxanthin and skin health

The skin is often exposed to sunlight, which contains the hazardous ultraviolet (UV) irradiation. Excessive exposure of unprotected skin to sunlight results in erythema, sunburn and can lead to photo-induced inflammation, immunosuppression, aging and even carcinogenesis of skin cells (melanoma). Pre-clinical studies illustrated that classic dietary antioxidants, such as α-tocopherol, vitamin C, or β-carotene could reduce such damage. Protecting skin from reactive oxygen species (ROS) nowadays motivates the inclusion of antioxidants in many cosmetics. The characteristics of Astaxanthin, administered orally and topically as superior antioxidant, motivated the research in exploring its potential in skin health and cosmetics.

The ability of Astaxanthin to protect rat kidney fibroblasts from UVA-induced oxidative stress (O'Connor and O'Brien, 1998) has led this Irish research group to test Astaxanthin's protective abilities in human skin fibroblasts as well. The scientists used an Astaxanthin-rich algal extract and examined its ability to protect against UVA-induced DNA alterations in human skin fibroblasts and human melanoma cells (Lyons and O'Brien, 2002). Human cells contain DNA molecules that are organized as chromosomes in the cell's nuclei. Incubation of unprotected cells under UVA radiation is known to induce alterations to the DNA, such as spontaneous breaks and degradation of the DNA polymers into shorter fragments. Indeed, extracting the DNA from cells exposed to UVA radiation and separating the different DNA molecules by gel electrophoresis showed the appearance of lightweight DNA fragments. This was not the case with Astaxanthin-incubated cells: the integrity of the chromosomal DNA was maintained, as if no irradiation at all had been applied. The algal extract already displayed protection against UVA-induced DNA damage with the equivalent of 10 μM Astaxanthin addition to both cell types. In human skin fibroblasts, exposure to UVA for two hours resulted in a significant induction of the enzyme superoxide dismutase (SOD) activity, coupled with a marked decrease in cellular glutathione (GSH) content. However pre-incubation (18 h) with 10 μM of Astaxanthin prevented UVA-induced alterations in SOD activity and GSH content.

Having shown that Astaxanthin is effective on melanoma cells, we shall now demonstrate how it inhibits their over-production of melanin, an important step in their carcinogenesis. This phenomenon is also known as hyper-pigmentation of the skin, in the form of suntan, stains or freckles. Arakane was looking at the activity of the enzyme tyrosinase in mouse melanoma cells, an important enzyme in melanin synthesis (Arakane, 2002). Mouse melanoma cells that were cultured with Astaxanthin for 3 days had a 60% lower amount of melanin than control cells, and the inhibitory effect was

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dose-dependent. While Astaxanthin failed to inactivate isolated tyrosinase *in vitro*, measurements on Astaxanthin-treated melanoma cell cultures showed decreased tyrosinase activity *in situ*. These results suggest an indirect antioxidative effect of Astaxanthin, probably through the inhibition of the auto-oxidation of the substrate for tyrosinase.

Animal models for skin health are not adequate, for the simple reason that unlike humans, the skin of most mammals is covered with fur. To this end, hairless mice are commonly used, as in the following experimental set (Arakane, 2002). Hairless mice were irradiated five times weekly with UVB irradiation for 18 weeks, routinely followed by topical treatment of their back skin with 0.1 ml of Astaxanthin (350 μM), or just vehicle. The researchers detected high wrinkle formation in the UVB-irradiated back skin of mice that received no Astaxanthin. The irreversible wrinkling phenomenon was significantly lower with the Astaxanthin- treated mice, as compared with the vehicle-treated mice. Scanning electron micrographs of the ultra structure of dermal collagen fiber bundles indicated that the application of Astaxanthin yielded remarkable maintenance of the bundle structures, accompanied by a reduction in wrinkles. This paper demonstrated the potential of post-irradiation topical treatment with Astaxanthin to reduce skin wrinkling, one of the most important targets of cosmetic treatments and products.

An additional approach for maintaining skin health is by oral administration of active ingredients and antioxidants. The group of Savoure and coworkers investigated the effect of UVA and UVB radiations on the skin of hairless mouse fed a diet supplemented with retinol, β-carotene or Astaxanthin (Savoure et al., 1995). They searched for UV-induced modifications in polyamine metabolism by measuring epidermal concentrations of free polyamines, useful markers for skin photo-damage. Mice irradiated with UVA + UVB had a higher level of the polyamine putrescine. Astaxanthin supplementation in the feed had a stronger inhibitory effect on putrescine accumulation than retinol, and successfully decreased the levels of two additional polyamines, spermidine and spermine. These results support the role of Astaxanthin as skin photo-protector both topically and orally.

Yet, studies in humans are irreplaceable. Astaxanthin's ability to suppress post UVB hyper-pigmentation in humans was already revealed in 1995 (Yamashita, 1995). The reddening (erythema) of our skin after exposure to UV irradiation is a leading cause for hyper-pigmentation and was found to be reduced by both synthetic and natural forms of Astaxanthin. Healthy adult men (7) were topically treated with Astaxanthin on their backs by occlusive dressing, 24 hours prior to UVB irradiation. The erythema grade was measured periodically and the hyper-pigmentation grade was measured one

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week after irradiation. Statistically significant suppression of hyper-pigmentation (lower melanin index) was measured in tests using natural Astaxanthin when compared to a control group. Synthetic Astaxanthin showed some suppression, though statistically non-significant. Furthermore, natural Astaxanthin demonstrated faster recovery times of the erythema index, suggesting anti-inflammatory properties that together with inhibition of melanin formation may contribute to the reduction of hyper-pigmentation. Since Astaxanthin does not absorb UVB light, the mechanism of erythema suppression might be related directly with oxidation of melanin that is produced in response to UV radiation.

A subsequent work was performed with 16 healthy women with dry skin, albeit Astaxanthin was given orally and combined with another novel antioxidant, tocotrienol (Yamashita, 2002). In this double blind clinical study, a noticeable improvement to skin condition was achieved, as reflected in elevated skin moisture content and reduced wrinkle appearance than in a placebo control group.

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(5) Astaxanthin and the immune response

The immune system is comprised of innate (natural) and acquired (adaptive) immunity. Acquired immunity includes the *lymphocytes*, highly active cells that constantly generate reactive oxidative species (ROS) as part of their normal cellular activity. Innate immunity includes the protection of the animal by phagocytizing and subsequently destroying antigens through an oxidative bactericidal mechanism termed *respiratory burst*. Phagocytosis of a foreign particle by a macrophage or neutrophil activates NADP oxidase, resulting in the production of a large quantity of superoxide anion (O_2^-) from molecular oxygen. The O_2^- is then rapidly converted to hydrogen peroxide (H_2O_2) by superoxide dismutase.

Neutrophils usually convert H_2O_2 to the highly potent bactericidal component *hypochlorite ions* (OCl^-), while macrophages generate other biological oxygen-derived free radicals such as *hydroxyl radical* (OH^-). While the ROS are produced as part of the killing mechanism, excessive phagocytic activity can lead to ROS-induced tissue damage (Chew and Park, 2004). Having these fighting cells inside our body is somewhat like playing with fire: Our own "firefighters" must be controlled. As a defense mechanism, the body produces a number of endogenous antioxidants capable of scavenging these harmful ROS to maintain an optimal oxidant-antioxidant balance, thereby maintaining normal cellular function and health. However, under conditions of high oxidative stress, the ability of these antioxidants to eliminate ROS is often exceeded and, therefore, dietary sources of antioxidants may be required.

The harmful affects of ROS are not unique to immune cells; all cell types are susceptible. However, immune cells are particularly sensitive to oxidative stress for several reasons. First, as described above, immune cells generally produce more ROS. Second, their plasma membranes contain a high percentage of polyunsaturated fatty acids, which are optimal oxidation targets. Third, immune cells rely heavily on cell-to-cell communications via cell membrane receptors. As will be discussed, Astaxanthin was shown to significantly influence the immune response in several animal models; much of the progress in this field is attributed to Jyonouchi and coworkers, who demonstrated the advantages of Astaxanthin in various immune responses.

In 1991, this group studied the immuno-modulating effects of β-carotene and Astaxanthin on mouse lymphocytes in *in vitro* culture systems. Antigen-stimulated lymphocyte proliferation normally occurs in lymphoid tissues. However, the ability of isolated lymphoid cells to proliferate when cultured in the presence of certain mitogens has given the researchers an important tool to assess both T and B cell function *in vitro*. In the presence of Astaxanthin, isolated B cells from the thymus (Jyonouchi et al., 1991) or from

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the spleen (Okai and Higashiokai, 1996) incorporated more materials from the culture media, and could hence accelerate their proliferation rate.

Yet, the more noteworthy immune effect of Astaxanthin is its enhancement of antibody production. Astaxanthin, yet not β-carotene, induced enrichment in antibody-forming cells such as plaque-forming cells and immunoglobulins (Ig) M and G in response to sheep red blood cells. Production of Ig has traditionally been used to assess B cell function in a humoral immune response. B cells produce Ig that circulates freely to protect the body against foreign materials. The Ig serve to neutralize toxins, immobilize certain microorganisms, neutralize viral activity, agglutinate microorganisms or antigen particles, and precipitate soluble antigens. B cell function requires the help of T-helper cells.

A follow-up study showed these effects *in vivo,* and also demonstrated that Astaxanthin could partially restore humoral immune responses in old mice (Jyonouchi et al., 1994). *In vivo* antibody production in response to T-dependent antigens was significantly enhanced by lutein, Astaxanthin, and β-carotene. The numbers of Ig M- and G-secreting cells also increased *in vivo* with the administration of these carotenoids when mice were primed with T-dependent antigens. Among these three carotenoids, only Astaxanthin significantly restored the antibody production of old mice. Depletion of T-helper cells prevented the enhancement of antibody production by lutein and Astaxanthin, suggesting that the mechanism by which these carotenoids act involves both T and B cells. The augmentation in the number of Ig-secreting cells was not restricted to one antigen, as similar >100% increases were reported following application with other foreign proteins from rabbits and pigeons (Jyonouchi et al., 1995a).

The effect of carotenoids on *in vitro* Ig production by human peripheral and cord blood cells was then examined in humans. Blood samples from adult volunteers and full-term newborn babies were cultured with and without carotenoid supplementation, and then were stimulated by T-dependent stimulant. Astaxanthin enhanced IgG, A, and M production in response to T-dependent antigens and a T-dependent stimulant, while the effect of β-carotene was negligible (Jyonouchi et al., 1995b).

A third measure of immune response is *cytokine production level*. Cytokines are soluble molecules that mediate cell-to-cell interactions, and are commonly produced by T-helper (Th) cells from subsets 1 and 2. The Th1 cells mediate cytotoxic and local inflammatory reactions, and therefore play important roles in combating intracellular pathogens including viruses, bacteria, and parasites. Th2 cells are more effective in humoral immunity, i.e.,

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they stimulate B cells to proliferate and produce antibodies against free-living microorganisms.

A normal immune response requires a balance between the Th1 and Th2 subsets. Over-production of the Th1 cytokine interferon-γ was successfully repressed (by 50%) only in Astaxanthin-cultured Th1 cells, an effect which was not seen under zeaxanthin, lutein, or lycopene (Jyonouchi et al., 1996). This repressing effect is of major importance, since it demonstrates how Astaxanthin can be useful in conditions of autoimmunity. In another study on mouse cell culture, Astaxanthin enhanced the release of interleukin-1, another cytokine molecule, better than any other carotenoid tested (Okai and Higashiokai, 1996).

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(6) Astaxanthin and inflammation

One of the ways in which an immune response becomes a pathological condition is inflammation. Severe inflammation, such as that in Crohn's disease and ulcer disease, involves the action of many ROS. These toxic molecules are released by phagocytic leucocytes, not only inducing oxidative stress, but also stimulating the expression of inflammatory genes in endothelial cells, which in turn aggravates the inflammation. Therefore, molecular effectors with anti-inflammatory properties are highly valuable. Astaxanthin exhibited such properties in several studies reviewed below.

As mentioned above, M. Kurashige et al (1990) managed to show that carrageenan-induced inflammation of a rat's paw was significantly inhibited by administration of Astaxanthin. A more recent study investigated the efficacy of Astaxanthin in lipopolysaccharide-induced inflammation of a rat's eye (Ohgami et al., 2003), a study briefly discussed in the eye health section. *Lipopolysaccharide* is a natural compound characteristic to the bacterial cell wall, and in this study, it induced *uveitis*, a specific inflammation of the eye characterized by the release of *cytokines* (soluble molecules that mediate cell-to-cell interactions) such as necrosis factors and interleukins, as well as inflammatory mediators including nitric oxide (NO) and prostaglandin E2, that are synthesized by inducible NO synthase. Astaxanthin suppressed the development of uveitis in a dose-dependent fashion. In addition, investigation of this inflammation in a mouse macrophage cell line showed that Astaxanthin decreased NO production, inducible NO synthase activity, and prostaglandin E2 and tumor necrosis factor-α production *in vitro* in a dose-dependent fashion. These results were confirmed in a more extensive work (Suzuki et al., 2005), described in the eye health section of this review. In both studies, the researchers concluded that Astaxanthin reduces ocular inflammation by down-regulating pro-inflammatory factors and by inhibiting the nuclear factor (NF)-kappaB)-dependent signaling pathway.

Still, Astaxanthin's anti-inflammatory molecular action and mechanism have not been elucidated. The best attempt to understand this mechanism was carried out in a similar macrophage cell line system (Lee e al., 2003) in which Astaxanthin was found to inhibit the expression and formation of the aforementioned pro-inflammatory mediators and cytokines in both lipopolysaccharide-stimulated cells and primary macrophages. Astaxanthin suppressed the serum levels of NO, prostaglandin E2, tumor necrosis factor-α, and interleukin-1 beta in lipopolysaccharide-administrated mice. Astaxanthin inhibited NF-kappaB activation as well as NO synthase promoter activity in lipopolysaccharide-stimulated cells. NF-KappaB has a seminal role in immunity, as it activates pro-inflammatory genes encoding for NO synthase, tumor necrosis factor-α, and several interleukins. Astaxanthin directly

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inhibited the intracellular accumulation of ROS in lipopolysaccharide-stimulated cells as well as H_2O_2-induced NF-kappaB activation and NO synthase expression. These results suggest that Astaxanthin inhibits inflammatory mediator production by blocking NF-kappaB activation and as a consequent suppression of IkappaB kinase activity and IkappaB-alpha degradation.

This recent breakthrough in the exploration of Astaxanthin's role in inflammation is important, though still far from explaining the whole picture. As an NF-kappaB inhibitor, Astaxanthin shows promising clinical potential in treating inflammatory diseases. Gastric ulcer is one of the most common among these diseases, and several studies were dedicated specifically to this pathological condition and its Astaxanthin answers, as will be described in the following chapter.

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(7) Astaxanthin and gastric ulcer

Helicobacter pylori is a Gram-negative pathogen colonizing the human gastric epithelium, causing type B gastritis, peptic ulcer disease, and gastric cancer. The pathogenesis of this infection is partly due to the immunological response. In the infected gastric mucosa of mice and humans, the immune response is polarized to a T-helper1 (Th1) cell-mediated response with release of a specific cytokine, which in turn activates phagocytic cells and contributes to mucosal damage. Low gastric tissue antioxidant levels are believed to increase the risk of developing these painful diseases, and hence Astaxanthin was repeatedly tested for its inhibitory effect.

Although gastric ulcer treatment with Astaxanthin has been under investigation in mammals for only seven years at this writing, the encouraging results have attracted several leading research teams in Sweden, Denmark, Japan, and Korea. The pioneers were the Scandinavians: In 1999 they published that algal cell extract-containing Astaxanthin reduces gastric inflammation and bacterial load in *H. pylori*-infected mice (Bennedsen et al., 1999). They further found that these changes are associated with a shift of the T-lymphocyte response from a predominant Th1-response dominated by interferon- γ to a Th1/Th2-response with interferon- γ and interleukin-4. Such a switch from a Th1-response to a mixed Th1 / Th2-response during an ongoing infection had not been reported previously, and proves again the tremendous potency of Astaxanthin.

As discussed in the previous chapters of this review, the inflammatory response is greatly influenced by the delicate balance between the cytotoxic actions of interferon- γ and the humoral actions of interleukin-4. This effect was previously reported in general inflammation conditions (Jyonouchi et al., 1996), and here it inhibits the gastric ulcer. Similar inhibition of the disease was found in *H. pylori*-infected guinea pigs (Sjunnesson et al., 2001). In the latter study, Astaxanthin was supplemented in combination with Vitamins A, C, and E, and showed even better inhibition.

Wang et al showed that better treatment against gastric ulcer could be achieved using a combination of Astaxanthin and Vitamin C (Wang et al., 200). Six-week-old mice were infected with the mouse-passaged *H. pylori* strain. At two weeks post-inoculation, mice were treated orally once daily for 10 days (i) with various doses of algal Astaxanthin, (ii) with a control meal (meal containing no Astaxanthin), or (iii) with Vitamin C (400 mg/kg). Five mice from each group were sacrificed one day after the cessation of treatment, and the other five were sacrificed 10 days after the cessation of treatment. Culture of *H. pylori* and determination of the inflammation score of the gastric mucosa were used to determine the outcome of the treatment. Mice treated

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with Astaxanthin-rich algal meal or Vitamin C showed significantly lower colonization levels and lower inflammation scores than those of untreated or control-meal-treated animals at one day and 10 days after the cessation of treatment. The healthiest animals were those given the higher dose of Astaxanthin-rich algal meal (containing 100 mg / kg Astaxanthin). Additionally, lipid peroxidation was significantly decreased in mice treated with the Astaxanthin-rich algal meal and Vitamin C compared with that of mice not treated or treated with the control meal.

A more recent study expanded on the meal treatment (Nishiwaka et al., 2005). β-carotene and Astaxanthin prepared from three different sources, namely the alga *Haematococcus*, the yeast *Phaffia*, and synthetic Astaxanthin, were used in these experiments. Rats given Astaxanthins or β-carotene prior to stressing were appreciably protected against the evolution of gastric ulcerations compared to control rats. Ulcer indexes were particularly lower in the rat group fed *Haematococcus*-extracted Astaxanthin than those of the other groups.

As demonstrated in the latter work, gastric ulcer can be an indirect result of stress (induced by starvation and low environment temperatures) as well as of direct inoculation with pathogenic *H. pylori.* Additional causes include ethanol and the drug naproxen, which have been found to induce ulcerative gastric lesion in humans. The *in vivo* protective effect of Astaxanthin was tested against ethanol-induced (Kim et al., 2005a) and naproxen-induced (Kim et al., 2005b) gastric mucosal injury in rats. The rats were treated with ethanol or naproxen for three days after pretreatment with two doses of Astaxanthin (5 and 25 mg / kg of body weight) for three days, while the control rats received only the inducing factor for three days. In both induced conditions, the oral administration of Astaxanthin showed significant protection against gastric lesion and inhibited elevation of the lipid peroxide level in gastric mucosa. In addition, pretreatment with Astaxanthin resulted in a significant increase in the activities of radical scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. A histological examination clearly indicated that the acute gastric mucosal lesion nearly disappeared after pretreatment with Astaxanthin.

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(8) Astaxanthin and the cardiovascular system

Cardiovascular diseases have been a major concern for years in Western countries. In the US alone, more than 2.4 million die each year from cardiovascular diseases, making it America's number one killer. Cardiovascular diseases include heart disease, stroke, high blood pressure, congenital heart defects, hardening of the arteries, and other diseases of the circulatory system. Within the last decade, there have been many reports in the literature documenting a beneficial effect of various carotenoids in the cardiovascular system. The past few years have seen a vast increase in experimental results for the inhibitory role of Astaxanthin in many forms of cardiovascular disease. In this chapter, we describe these exciting findings according to the specific disorders.

Arteriosclerosis is a process in which plaque builds up in the artery walls, reducing blood flow. Total blockage of the narrowed artery results in a heart attack, and indeed most heart attacks are related to arteriosclerosis. Susceptibility to arteriosclerosis is determined by a combination of genetic and environmental factors, including diet, especially a diet high in cholesterol. High blood levels of LDL-cholesterol (Low Density Lipoprotein, the "bad" cholesterol) are associated with an increased risk of arteriosclerosis. On the other hand, HDL (High Density Lipoprotein, the "good" cholesterol) blood levels are inversely correlated with coronary heart disease and are pinpointed as protection against arteriosclerosis. In the blood plasma, LDL is usually not oxidized, and when its oxidation does occur, it is believed to contribute to the development of arteriosclerosis. Therefore, supplementation with an antioxidant might reduce the risk of arteriosclerosis, as indicated by epidemiological and clinical data.

A glimpse into the preventative effect of Astaxanthin in arteriosclerosis was published in 1992. In an animal model, Astaxanthin (but not β-carotene) supplementation for 30 days led to an increase in blood levels of HDL (Murillo, 1992). In addition, Miki et al (1998) demonstrated the antiatherogenic anti-oxidative mechanism *in vitro* with humans. Human subjects ingesting daily dosages as low as 3.6 mg of Astaxanthin per day for two consecutive weeks had lower levels of induced *in vitro* oxidation of their LDL cholesterol fraction. Supplementation with Astaxanthin reduces the level of oxidized LDL, and hence prevents plaque formation on the walls of our blood vessels.

The effects of Astaxanthin on *in vitro* and *ex vivo* LDL oxidation were measured in a later study (Iwamoto et al., 2000). The oxidation of LDL was measured in a 1 ml reaction system consisting of increasing concentrations of

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Astaxanthin and constant LDL concentration (70 µg / ml protein). Astaxanthin dosing dependently significantly prolonged oxidation lag time compared with the control. For the *ex vivo* study, 24 volunteers (mean age 28.2 years) consumed Astaxanthin at various doses for 14 days. No other changes were made in the diet. Fasting venous blood samples were taken at days 0 and 14. Indeed, LDL lag time was longer respectively (5.0, 26.2, 42.3, and 30.7%) when compared with day 0, after consuming Astaxanthin at doses of 1.8, 3.6, 14.4, and 21.6 mg / daily for 14 days; no difference was observed in the oxidation of LDL between day 0 and day 14 in the control group.

In a recent study, the effect of Astaxanthin combined with two more compounds was investigated in a model of experimental arteriosclerosis provoked in the rabbit by atherogenic cholesterol-enriched feed. Although Astaxanthin was not individually tested in this case, its net effect can be inferred from comparison of the treatments with or without it. Atherogenic cholesterol-enriched feed is known to release free radicals, which in turn enhance lipid peroxidation in the feeding animals. The researchers traced the increase of *blood malondialdehyde*, a tracer of lipid peroxidation, and found that Astaxanthin was able to markedly reduce the prevalence of this compound in the blood. Yet the most remarkable effect of Astaxanthin was its ability to prevent plaque formation on the aorta wall. The lipid infiltration of the aortas of Astaxanthin-fed rabbits was reduced by 92%, whereas without Astaxanthin (but supplemented with the other two compounds), the lipid infiltration of the aorta was reduced by only 33%.

Having shown its protective effect against arteriosclerosis, additional studies focused on Astaxanthin's role in another cardiovascular disorder, hypertension (high blood pressure). Spontaneously hypertensive rats were chosen as an experimental model to test the effects of Astaxanthin on this disorder (Hussein et al., 2005a). The rats were orally administered Astaxanthin (50 mg / kg) for 14 days, then examined for a series of cardiovascular indices. The supplementation with Astaxanthin induced a significant (up to 10% reduction) in arterial blood pressure. This reduction was repeated in a second rat strain, stroke-prone spontaneously hypertensive rats, which also presented a significant delay effect in the incidence of stroke. By day 4 of the post-treatment period, the stroke rate was 60% in the control and lower Astaxanthin dose (5 mg / kg) groups, whereas the higher Astaxanthin dose (50 mg / kg) group did not show any sign of stroke; on day 14, the higher Astaxanthin dose (50 mg / kg) group showed a moderate incidence of stroke (40%) compared to the high incidence in the control group (80%).

Both blood pressure and stroke are predominantly affected by the sensitivity of the blood vessels, meaning the vascular reactivity. The contraction of blood vessels in both rats and humans is a highly regulated

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process, involving degradation of nitric oxide (NO) as a main signaling step. To that end, rat aortic ring preparations were studied *in vitro*. Astaxanthin induced vascular relaxation mediated by NO, as well as reducing vascular contraction, both in dose-dependent fashion. Based on these results, the scientists hypothesized that the vascular relaxation effect might result from Astaxanthin's anti-oxidative properties, presumably its superoxide scavenging effects in preventing superoxide-induced NO degradation, which would thus prolong its half-life and consequent vascular relaxation. This breakthrough hypothesis was verified in a second set of experiments by the same Japanese group (Hussein et al., 2005b), in which the scientists showed that Astaxanthin initiated a significant modulatory effect on NO-induced vascular relaxation. In addition, Astaxanthin demonstrated a significant effect on the blood fluidity of treated hypertensive rats, suggesting that its inhibitory influence on blood pressure and stroke incidence involve more than one mode of action. This blood pressure-reducing property makes Astaxanthin a potent natural hypertension solution for people all over the world.

Yet, the most sensitive part of the cardiovascular system is the heart itself. Whether directly or indirectly, it is most often the heart that fails, putting the lives of so many at risk. We cannot forget that the heart, being the blood pump, is by itself a muscular tissue that needs blood supply for its uninterrupted function. Therefore, failure to supply blood to the heart can lead to myocardial infarction, one of the major causes of stroke. In recent years, a novel Astaxanthin derivative (Disodium Disuccinate Astaxanthin; Cardax) was tested in this context in three different models. Astaxanthin produced dose-related reductions in myocardial infarct size (IS) in rats (Gross and Lockwood, 2004). The animals were administered intravenously at any of three doses (25, 50, and 75 mg / kg) on four consecutive days, followed by the acute infarct size study on day 5. Thirty minutes of occlusion of the left anterior descending coronary artery was followed by two hours of reperfusion prior to sacrifice. Maximum salvage occurred at the highest dose (75 mg / kg) tested, and was manifested as a 56% reduction in IS. Infarct size and myocardial salvage were significantly, linearly correlated with plasma Astaxanthin levels at the end of reperfusion.

In a follow-up study, (Gross and Lockwood, 2005), the researchers used a more relevant large animal model, the dog, and studied the effect of administering Cardax intravenously either acutely two hours prior to occlusion, or for four consecutive days at 50 mg / kg as in the rat model. In all groups, dogs were subjected to 60 min of left anterior descending coronary artery occlusion and three hours of reperfusion. Infarct size (IS) was 21% in controls and was reduced to 11% (47.3% salvage; $p < 0.01$) in dogs treated only once at two hours prior to occlusion, and 7% (68.4% salvage; $p < 0.001$) in dogs treated for four consecutive days. Amazingly, in the chronic treatment

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group, two of the three dogs with plasma concentrations of Astaxanthin above 1 μM had 0% IS, or 100% cardio-protection.

In parallel to the canine model, the same protocol was applied in a rabbit model as well (Lauver et al., 2005). Again, administration of Astaxanthin significantly reduced the myocardial IS, this time by 51%. These results suggest that Astaxanthin has marked cardio-protective properties in a variety of mammals. Thus, Astaxanthin may be a novel and powerful new means to prevent myocardial injury and / or necrosis. Such situations are frequently a result of elective and / or urgent cardiac surgical interventions, such as coronary angioplasty and stenting, as well as coronary artery bypass surgery.

The above-described studies indicate that Astaxanthin is beneficial for people suffering serious heart disease. However, is Astaxanthin useful for the healthy heart as well? One interesting paper suggests an affirmative reply to this question. In this study (Aoi et al., 2003), the effect of dietary supplementation with Astaxanthin on oxidative damage induced by strenuous exercise in mouse hearts was investigated. Mice were divided into groups: rested control, intense exercise, and intense exercise with Astaxanthin supplementation. After three weeks of exercise acclimation, both exercise groups ran on a treadmill at 28 m / min until exhaustion. Exercise-increased biochemical markers in the heart were blunted only in the Astaxanthin group. In addition, the researchers measured the exercise-induced activity of the plasma enzymes creatine kinase and myeloperoxidase in the heart, and again documented a lowering effect by Astaxanthin. These are the first indications for Astaxanthin's role in improving cardiovascular fitness, as expressed under strenuous exercise.

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(9) Astaxanthin and cellular health

Homeostasis, or the capacity of living cells to maintain their internal environment, is largely attributed to the existence of undamaged membranes in their fringes. Astaxanthin's superb capacity in protecting cellular membranes is believed to derive from its ability to protect both the inner part and external surface of membranes against oxidation, as discussed above. A physical chemistry technique based on singlet oxygen luminescence at about 1,270 nm, and a biological cell membrane technique were used to study the quenching of singlet oxygen by four carotenoids bound to the surface of human lymphoid cells (Tinkler et al., 1994). All of the carotenoids studied exerted a beneficial effect in cell protection, with lycopene and Astaxanthin emerging as the leading membrane conservers.

Membranes do not only separate cells from their surrounding milieu; they also play a crucial role in the communication between each cell and its environment. Many cell membranes contain pores (called cell gap junctions) that permit cell-to-cell communications needed to modulate cell growth and, in pathological cases, limit expansion of cancer-infected cells. Carotenoids are active inducers of communication between cells at the gap junctions. Much effort has recently been invested in investigating how Astaxanthin is involved in these processes.

Gap junctions, also known as *connexons*, are formed by the assembly of *trans-membrane connexin proteins*, and have multiple functions including the coordination of cell responses. Most human tumors are deficient in gap junctional intercellular communication (GJIC), and the restoration of GJIC by forced expression of connexins reduces indices of neoplasia (tumor tissue growth). The expression of *connexin 43*, the most widely expressed connexin family member, is up-regulated by cancer-preventative retinoids and carotenoids, which correlates with the suppression of carcinogen-induced transformation in T1/2 cells (Hix et al., 2004). When delivered to mouse embryonic fibroblast T1/2 cell cultures, Astaxanthin up-regulated the expression of connexin 43 protein. Moreover, Astaxanthin did not require protein synthesis for the induction of connexin 43 mRNA, indicating direct transcriptional activation (Vine et al., 2005). However, the exact regulatory pathway is still being debated.

Another recent study suggests that Astaxanthin affects gap junction function by changing the phosphorylation pattern of connexin 43, rather than activating its transcription (Daubrawa et al., 2005). In this later work, gap junctional intercellular communication (GJIC) was even diminished by

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Astaxanthin at levels > 0.1 µmol/L, in contrast to the reported inducing activity of Astaxanthin. Incubation of human skin fibroblasts with Astaxanthin led to a change in the phosphorylation pattern of connexin 43, shifting from higher to lower phosphorylation states. Until the exact action mode is deciphered, we can declare only that Astaxanthin modifies gap junctional intercellular communication.

The cellular role of Astaxanthin is not limited to the membrane alone. One particular cell organelle produces large quantities of free radicals that must be neutralized to maintain its proper function. The multiple oxidative chain reactions in the mitochondria, which generate the energy required by the cell, damage this organelle in a cumulative fashion. This very process is believed to be a major cause of tissue aging and cell death. The efficacy of Astaxanthin in preventing *in vitro* peroxidation of mitochondria in rat liver cells was as high as 100 times that of Vitamin E (Kurashige et al., 1990). This efficacy and its membrane-protective capacities highlight the unique function of Astaxanthin in helping to preserver cellular health, and its promising potential in the fight against aging.

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(10) Astaxanthin and anti-cancer activity

The anti-cancer activity of carotenoids has been the focus of much attention since epidemiological reports of an association between low systemic levels of certain carotenoids and various types of cancer. For instance, while men with the lowest plasma β-carotene levels had an increased risk of prostate cancer, when supplemented with carotenoids, their risk decreased by 36% (Maher, 2000). Many animal studies have manifested the anti-cancer properties of various carotenoids, including Astaxanthin. This chapter presents an up-to-date review of the ample scientific activity in this field.

Having started with effect on risk of prostate cancer, we now have supportive evidence for the beneficial involvement of Astaxanthin in this type of cancer treatment (Anderson, 2005). The potential involvement of Astaxanthin in such carcinogenic processes within a living tissue or a cell culture is by no means straightforward. There are many direct and indirect mechanisms and biochemical pathways in which Astaxanthin has been shown to function or may function. Yet, in the case of prostate cancer, a preliminary pathway has been suggested. According to the working model, Astaxanthin induces inhibition of the enzyme 5alpha-reductase, which in turn restrains the growth of prostatic carcinoma cells. In this study, Astaxanthin's effect was assayed *in vitro* using human cell line. The results show that Astaxanthin caused 98% inhibition of 5alpha-reductase, and a nine-day treatment of prostatic carcinoma cells with Astaxanthin produced a 38% decrease in growth. This observation may provide a partial explanation of the previously reported decreased risk of prostate cancer with carotenoid supplementation.

A nearby organ with high rates of cancer incidence is the bladder, the site of induced carcinogenesis in mice tested for its prevention by Astaxanthin and canthaxanthin (Tanaka et al., 1994). Mice were administered the carcinogen in their drinking water for 20 weeks, followed by Astaxanthin and canthaxanthin supplementation for additional 20 subsequent weeks. At the end of the study (week 41), the incidences of tumor cells in the bladder of mice treated with the carcinogen and Astaxanthin or canthaxanthin were lower than those of mice given the carcinogen alone; in particular, Astaxanthin administration after exposure to the carcinogen significantly reduced the incidence of bladder cancer (transitional cell carcinoma) ($P < 0.003$).

Appearance of neoplasms is not the first step in the onset of urinary cancer. A primary step in the onset of bladder cancer is cell proliferation, which is typically characterized by in rise in the number of specific proteins in the nuclear region. In this study, the scientists were able to stain these proteins with silver and then count them under the microscope. Treatment with Astaxanthin decreased the number of the silver-stained proteins in the nuclei

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of cells from transitional epithelium exposed to carcinogen. This result demonstrates that Astaxanthin's mode of action coincides with very early stages of carcinogenesis.

While prostate cancer is a merciless killer of human males worldwide, human females are under the looming threat of breast cancer. The anticancer activities of β-carotene, Astaxanthin, and canthaxanthin against the growth of mammary tumors were studied in female mice (Chew et al., 1999). The mice were fed a diet containing each of the three carotenoids; after three weeks, all of the mice were inoculated with about one million tumor cells into the mammary fat pad. At the end of the experiment, Astaxanthin was found to be the best inhibitor of tumor growth in mammary glands. Astaxanthin tumor inhibition was found to be dose-dependent, unlike that of β-carotene canthaxanthin. Notably, Astaxanthin was also the only carotenoid to show up in high concentrations in the blood plasma, as well as demonstrating lower lipid peroxidation activity in the tumors.

Another cancer type successfully inhibited by Astaxanthin in animals was liver cancer (Gradelet et al., 1997, and 1998). Upon initiation of liver cancer in male rats by aflatoxin B1, supplementation of Astaxanthin reduces the carcinogenicity of this toxic molecule. Astaxanthin treatment reduced the number of cancer cells by 60-80%, and decreased their size by 25-30%, while lycopene or excess Vitamin A did not show a preventative effect. Astaxanthin supplementation lowered the incidence of DNA Single Strand Breaks, and deviated the aflatoxin B1 metabolism toward detoxification pathways. Similar effects were detected in two different types of digestive system cancer: colon cancer (Tanaka et al., 1995a) and oral cancer (Tanaka et al., 1995b). In both studies, Astaxanthin was administered during the carcinogen treatment or following it; interestingly, Astaxanthin halted the development of lesions in the oral cavity and the large intestine of rats both during the initiation of oral cancer and at the post-initiation phase. The authors concluded that Astaxanthin can serve as a possible chemopreventer for colon and oral carcinogenesis.

Cancer metastasis can be inhibited by Astaxanthin, as well as certain intrinsic factors. For example, natural killer cells of the immune system are involved in anti-tumor activity and the inhibition of cancer. Integration of these two facts, in addition to the previously shown influence of Astaxanthin on immune cells led a Japanese research team to test the possibility of immunological mechanism in cancer prevention by Astaxanthin (Kurihara et al., 2002). When mice were treated with restraint stress alone, the total number of spleen cells, and the level of natural killer cell activity per spleen, were reduced to a minimum level on day 3. The stress also caused a significant increase in the lipid peroxidation of liver tissue. Astaxanthin supplementation improved the immunological dysfunction induced by restraint stress. In

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addition, metastatic nodules were observed in the livers of the mice on day 12, after inoculation of the animals with mastocytoma cells. Hepatic metastasis was promoted further by restraint stress when applied on day 3, before the inoculation of mastocytoma cells. Daily oral administration of Astaxanthin markedly attenuated the promotion of hepatic metastasis induced by restraint stress. These results suggest that Astaxanthin improves anti-tumor immune responses by inhibiting stress-induced lipid peroxidation.

Immune cell protection by Astaxanthin as a mode of cancer prevention was considered even earlier by Jyonouchi et al (2000), who determined the effects of dietary Astaxanthin on tumor growth and tumor immunity against transplantable, chemically induced fibro sarcoma cells. These tumor cells express a tumor antigen that induces T cell-mediated immune responses in mice. The animals were fed Astaxanthin mixed in a chemically defined diet, starting day zero, one weeks, and three weeks before subcutaneous inoculation with a minimal tumorigenic dose of tumor cells. Three weeks after inoculation, tumor size and weight were determined, along with other relevant parameters. The Astaxanthin-fed mice had significantly lower tumor size and weight than controls when supplementation was started one and three weeks before tumor inoculation. This anti-tumor activity paralleled higher cytotoxic T lymphocyte activity and interferon-γ production in the Astaxanthin-fed mice. Cytotoxic T lymphocyte activity by tumor-draining lymph node and spleen cells was highest in mice fed Astaxanthin for three weeks before inoculation. When the Astaxanthin-supplemented diet was started at the same time as tumor inoculation, none of these parameters were altered by dietary Astaxanthin except interferon-γ production by spleen cells. These results indicate that dietary Astaxanthin suppressed the tumor cell growth *via* stimulation of the immunity against the specific tumor antigen.

Moreover, this study is the first to define the time window of Astaxanthin anti-cancer activity: In order to help the body fight tumor cells, Astaxanthin has to be taken before a critical mass of tumor cells builds up in a certain site. We must not forget that in this study, animals were tested under extreme conditions, as the disease onset was simultaneous with its peaking critical mass, since mice were directly inoculated with tumor cells at double the amount required for cancer induction. The human analogous situation is an individual suffering acute, late-stage cancer with very low cure options.

Prevention is always better than cure, and this time-tested adage is particularly relevant in the case of cancer where cure, if at all possible, is frequently associated with highly cytotoxic agents and / or invasive procedures. In recent years, a novel mechanism for the anticancer activity of certain carotenoids, including Astaxanthin, was suggested (Bertram and Vine, 2005). The model is based on the fact that virtually every human tumor is

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deficient in gap junctional intercellular communication (GJIC), and the restoration of GJIC by forced expression of proteins (connexins) reduces indices of neoplasia. The expression of connexin 43 is up-regulated by cancer-preventive retinoids and carotenoids, which correlates with the suppression of carcinogen-induced transformation in T1/2 cells (Hix et al., 2004). When delivered to mouse embryonic fibroblast T1/2 cell cultures, Astaxanthin up-regulated expression of connexin 43 protein and increased formation of connexin 43 immunoreactive plaques in regions of the plasma membrane consistent with localization of gap junctions. Astaxanthin significantly up-regulated GJIC as demonstrated by Lucifer Yellow dye transfer after microinjection. Enhanced expression of connexin 43 and increased GJIC result in the inhibition of *in vitro* malignant tumor growth, as well as growth reduction of human tumors in external grafts.

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(11) Astaxanthin and liver function

The human liver is an organ, frequently referred to as the "integrated laboratory" within the human body. Indeed, intense catabolism and anabolism take place in this complex organ. Among the many functions of the liver are active oxidation of lipids to produce energy, detoxification of contaminants, and destruction of pathogenic bacteria, viruses, and dead red blood cells. These functions can lead to significant release of free radicals and oxidation byproducts, which in turn endanger the liver cells themselves. Evidence of oxidative stress has been detected in almost all of the clinical and experimental conditions of chronic liver disease with differing etiology and fibrosis progression rates, often in association with decreased antioxidant defenses (Parola and Robino, 2001). Therefore, the liver has evolved various mechanisms that protect it against oxidative damage.

The liver's defense mechanisms can be divided into three different levels, in each one of which the involvement of Astaxanthin has been shown to be beneficial. The most basic defense level is the direct oxidation target level. Lipid peroxidation was measured in mitochondria from rat liver cells, with or without the presence of strong antioxidants. Astaxanthin was found to be much more effective than Vitamin E at protecting the mitochondria, as it significantly suppressed the changes in their phospholipid components (Kurashige et al., 1990).

The second level of liver protection is at the detoxification level. Astaxanthin induces xenobiotic-metabolizing enzymes in rat livers (Jewell and O'Brien, 1999; Gradelet et al., 1996). Foreign molecules (*xenobiotic*) are metabolized in the liver as part of its detoxifying action. Xenobiotic metabolizing enzymes can be divided into phase I and phase II enzymes. The majority of phase I reactions are catalyzed by one enzyme system, *cytochrome P450 monooxygenase*. The cytochrome P450 system is actually a collection of isoenzymes, which catalyze various types of oxidation reactions. Phase II reactions, also known as *conjugation reactions*, involve the addition of a polar group to the foreign molecule. Among phase II enzymes, glutathione conjugation by the enzyme glutathione-S-transferase (GST) is of particular importance, since it is often involved in the removal of reactive intermediates. One group (Astorg et al., 1997; Gradelet et al., 1996) has reported that canthaxanthin and Astaxanthin are excellent inducers of cytochrome P450 1A1 and 1A2 activity in livers of male rats.

An Irish group was studying the effect of 16 days' intake of a 300 mg carotenoids / kg diet on xenobiotic metabolizing enzymes in the livers, lungs, kidneys, and small intestines of male rats (Jewell and O'Brien, 1999). Only

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Astaxanthin and canthaxanthin intake inferred significant increase of all these metabolizing enzymes of the liver, an up to 55-fold increase, suggesting that both carotenoids are potent inducers of contaminant oxidation.

How does this induction activity affect the metabolism of Astaxanthin itself in the liver? To that end, HPLC and gas chromatography-mass spectrometry analyses were used in the characterization of primary cultures of rat liver cells (hepatocytes). Within 24 hours, more than 50% of the Astaxanthin was metabolized and conjugated (Wolz et al., 1999). It was confirmed that Astaxanthin induces xenobiotic-metabolizing enzymes in rat livers *in vivo*. However, there were no differences in the metabolism of Astaxanthin in cultured hepatocytes from rats that were pretreated with Astaxanthin and thus with induced cytochrome P-450 systems, compared with control hepatocytes. Neither liver microsomes from Astaxanthin-pretreated nor control rats metabolized Astaxanthin. These results indicate that the cytochrome P-450 enzymes were not involved in the metabolism of Astaxanthin in rat hepatocytes; it was suggested that Astaxanthin was metabolized in primary cultures of rat hepatocytes independent of the xenobiotic-metabolizing enzymes induced by Astaxanthin.

As remarkable as these findings are so far, research has progressed even further. Investigation of Astaxanthin metabolism in primary cultures of human hepatocytes confirmed its role in induction of the major cytochrome P450 enzyme (Kistler et al., 2002). Results presented by this study also show four metabolites derived from radio-labeled Astaxanthin in cultured hepatocytes and human plasma from two volunteers who had taken 100 mg Astaxanthin orally 24 hours before blood collection. As in the rat model, Astaxanthin was identified in human hepatocytes as an inducer of several cytochrome P-450 enzymes, though not all. These results indicate that Astaxanthin induces detoxifying enzymes in human liver cells, which do not participate in Astaxanthin's degradation.

The modulation of xenobiotic-metabolizing enzymes by Astaxanthin has led the research French team of Astorg and Gradelet to test Astaxanthin’s potential role in a third level, namely prevention of chemical carcinogenesis. In two published works (Gradelet et al., 1997, and 1998), the French scientists show that upon initiation of liver cancer in male rats by aflatoxin B1, consumption of Astaxanthin reduces the carcinogenicity of this toxic molecule. As was described earlier in this review, the Astaxanthin treatment reduced the number of cancer cells by 60-80%, and decreased their size by 25-30%, while lycopene or excess Vitamin A did not show any preventative effect. In addition, Astaxanthin, canthaxanthin, and the enzymatic inducer 3-MC significantly reduced aflatoxin B1-induced DNA single-strand breaks. It was also revealed that Astaxanthin diverts the metabolism of aflatoxin B1 into

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the less genotoxic aflatoxin M1, and hence succeeds in the detoxification of the carcinogenic molecules.

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(12) Astaxanthin and central nervous system

The central nervous system is particularly susceptible to oxidative damage. Nerve tissue is rich in unsaturated fatty acids (which are sensitive to oxidation) and iron (which possess pro-oxidative properties). Additionally, the nervous system has strong metabolic activity, which releases reactive compounds and free radicals as by-products. The medical literature presents substantial evidence that oxidative stress is involved in the pathogenesis of major neurodegenerative diseases such as Alzheimer's, Huntington's, Parkinson's, and ALS. The nervous system's susceptibility to oxidative conditions and damage emphasizes the potential important role of antioxidants in its proper functioning.

As revealed in an aforementioned study (Tso and Lam, 1996), Astaxanthin is able to cross the Blood Brain Barrier in mammals, and can extend its antioxidant properties beyond that barrier. It is therefore a promising candidate for testing in many neurological diseases.

A recent animal study showed astonishing results regarding Astaxanthin's neuro-protective effects (Hussein et al., 2005). Male mice were subjected to transient cerebral ischemia induced by bi-lateral common carotid artery occlusion, a well-documented model for ischemia in humans. Two other groups of mice were administered Astaxanthin (55 and 550 mg / kg) one hour prior to the artery occlusion, and a fourth group underwent the operation, albeit without the artery occlusion, and hence served as sham. The researchers tested the mice's performance in the Morris water maze, in which they may escape the water onto a platform, an action requiring learning and memory skills. The time course of escaping to the platform was significantly reduced in Astaxanthin-fed mice, from 35 s in the control group, to 25 s in the 55 mg / kg group, and 15 s in the 550 mg / kg group, almost as low as the sham group, reaching the platform in 10 s only. At day 7, Astaxanthin-fed mice presented better memory skills as well. While control mice spent only 30% of the time at the quadrant where the platform used to be, mice fed with 550 mg / kg Astaxanthin, as well as sham mice, spent 50% of their time there. These results suggest that Astaxanthin may have beneficial effects in improving memory in vascular dementia.

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(13) Astaxanthin and the reproductive system

Infertility results from the synergistic coincidence of four major factors: genetic defect or constitution, life style factors, professional and environmental exposure and specific diseases related to the reproductive organs. Evidence has accumulated supporting the pivotal role of reactive oxygen species (ROS) in the pathogenesis of sperm dysfunction among men with infertility. Spermatozoa posses little defense against oxygen damage and are highly sensitive to ROS, inducing changes in the fatty acid composition of the sperm membranes and damage to sperm DNA. Due to the high concentration of polyunsaturated fatty acids (PUFA), spermatozoa membranes are highly vulnerable to oxidation, reducing the fluidity and fusogenic capacity of the membrane. Astaxanthin is a lipid soluble antioxidant with inhibitory effects of ROS activity and lipid peroxidation. Its potential involvement in semen quality was therefore assessed.

El Garem and coworkers evaluated the effect of supplementation with algal Astaxanthin on the semen quality of infertile male volunteers (El Garem et al. 2002). This double blind randomize trial included 20 couples suffering from infertility for at least 12 months and with diagnosed abnormal semen quality. The sub-fertile man received either 16 mg/day algal Astaxanthin, or identically packed placebo capsules during a period of three months, in addition to the conventional treatment as recommended by the World Health Organization (WHO). Following three months' treatment, the ROS activity in the semen decreased in the Astaxanthin group, while no change was observed in the placebo group. In addition, sperm motility and morphology was improved in the supplemented group. At the end of the treatment period, five couples out of ten successfully conceived in the Astaxanthin-supplemented group, compared to one out of ten couples in the placebo group. The researchers concluded that supplementation with Astaxanthin improved the quality of the spermatozoa, which is suggested to be the explanation for the increased frequency of conception.

A few years later, Comhaire and his coworkers performed a more detailed double blind, randomized trial design, looking at the effect of algal Astaxanthin supplementation on 30 men with infertility of at least 12 months, and female partners with no demonstrable cause of infertility (Comhaire et al 2005). The men received conventional WHO treatment and Astaxanthin (16 mg/day), or conventional WHO treatment and placebo for three months. The effects of the treatments on sperm parameters, reactive oxygen species (ROS), serum hormones including testosterone and Inhibin B and spontaneous or intrauterine insemination-induced pregnancies were evaluated. ROS and Inhibin B decreased significantly and sperm linear velocity increased in the Astaxanthin group (n = 11), but not in the placebo group (n = 19). The results

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of the zona-free hamster oocyte test tended to improve in the Astaxanthin group in contrast with the placebo group, though not reaching statistical significance. The pregnancy rate among the placebo cases, 10.5 %, was significantly lower compared with 54.5 % in the Astaxanthin group (P = 0.028). This study suggests a positive effect of Astaxanthin on sperm parameters and fertility, with more than fivefold increase in pregnancy rates.

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(14) Astaxanthin and diabetes

The incidence of Type 2 Diabetes Mellitus is increasing worldwide. Type 2 diabetes results from the interaction between a genetic predisposition and behavioral and environmental risk factors. Most people who develop this disease are resistant to insulin, the hormone produced by the pancreas that allows glucose to enter the cells of our body. Others simply cannot produce enough insulin to meet their bodies' needs. Although the genetic basis of this disease has yet to be fully understood, there is strong evidence that such modifiable risk factors as obesity and physical inactivity are the main non-genetic determinants of Type 2 Diabetes.

A number of experimental studies have suggested the involvement of reactive oxygen species (ROS) in the onset of Diabetes Mellitus and the development of diabetic complications. Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic beta cells and various forms of tissue damage in patients with Diabetes Mellitus. It was therefore suggested that powerful antioxidants such as Astaxanthin might elicit beneficial effects against the progressive destruction of pancreatic beta cells. A team of researchers from Kyoto University of Medicine has dedicated the last several years to the examination of this promising opportunity.

In 2002, the first encouraging results were summarized in an interesting paper (Uchiyama et al., 2002). The scientists used diabetic db/db mice, a well-known obese model of Type 2 Diabetes. In this mouse, hyperglycemia arises because of increasing insulin resistance and the subsequent insufficiency of beta cell compensation. For the control group, the scientists used their non-diabetic littermates, db/m mice. Astaxanthin treatment was started at six weeks of age, and its effects were evaluated at 10, 14, and 18 weeks of age by non-fasting blood glucose levels intra-peritoneal glucose tolerance test including insulin secretion, and beta-cell histology.

The non-fasting blood glucose level in the db/db mice was significantly higher than that of db/m mice, and the higher level of blood glucose in db/db mice was significantly decreased after treatment with Astaxanthin. The ability of islet cells to secrete insulin, as determined by the intra-peritoneal glucose tolerance test, was preserved in the Astaxanthin-treated group. In conclusion, these results indicate that Astaxanthin can exert beneficial effects on diabetes, with preservation of beta-cell function.

As mentioned above, ROS may be involved not only in the onset of type 2 Diabetes, but also in many diabetic complications. It is hence natural that the next step of this research endeavor was to focus on the effect of Astaxanthin on one of these common complications, namely nephropathy (Naito et al.,

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2004). Diabetic nephropathy is characterized by the enlargement of glomerular mesangium due to the accumulation of extra-cellular matrix proteins, and is a leading cause of end-stage renal disease. Clinical studies in subjects with Type 1 and Type 2 diabetes clearly link hyperglycemia to vascular complications, including diabetic nephropathy. In this study, the researchers examined whether chronic administration of Astaxanthin could prevent the progression of diabetic nephropathy induced by oxidative stress in mice. Again, female db/db mice were used, and non-diabetic db/m mice served as a control. After 12 weeks of treatment, the Astaxanthin-treated group showed a lower level of blood glucose as compared with the non-treated db/db group. The relative mesangial area in the Astaxanthin-treated group was significantly smaller than the non-treated db/db group. The increases in urinary albumin and DNA oxidation marker at 12 weeks of treatment were significantly inhibited by chronic supplementation with Astaxanthin. The results suggested that the antioxidative activity of Astaxanthin reduced oxidative stress on the kidneys and prevented renal cell damage. In conclusion, administration of Astaxanthin might be a novel approach for the prevention of diabetes itself, and with the progression of this disease, diabetes nephropathy as well.

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