

Astaxanthin Overview

Astaxanthin is a fat-soluble, oxygenated pigment called a xanthophyll and a member of the carotenoid family. It has a unique molecular structure that gives it powerful antioxidant function. It is extracted from salmon, microalgae, and Pfaffia, a yeast. Current research shows that due to astaxanthin's potent antioxidant activity, it may be beneficial in cardiovascular, immune, anti-inflammatory, and neurodegenerative diseases. Specifically it inhibits lipid peroxidation at the cell level; crosses the blood-brain barrier, effecting treatment of ocular, and neurodegenerative diseases such as glaucoma and Alzheimer's; provides significantly more antioxidant capacity than other carotenoids and antioxidants such as beta-carotene and Vitamin E; entraps free radicals by adding them to its long, double-bonded chain rather than donating an electron; stabilizes the cell membrane like a bridge because its polar end groups span the cell membrane, thus increasing its rigidity and mechanical strength; neutralizes singlet and triplet oxygen (de-charges) generated by UVA and UVB radiation and other sources; binds to a lipoprotein, an efficient transport vehicle, making it more bioavailable; increases immune system function including heightened production of antibody-secreting cells and Interleukin 2 and suppression of Interferon-gamma; inhibits reactive oxygen species that cause inflammation; enhances the antioxidant actions of Vitamin E and Vitamin C and encourages the release of Vitamin A from the liver when needed.

Astaxanthin has 100-500 times the antioxidant capacity of Vitamin E and 10 times the antioxidant capacity of beta-carotene. Many laboratory studies also indicate astaxanthin is a stronger antioxidant than lutein, lycopene and tocotrienols.

Dosage: The recommended dosage of 1 mg twice per day is similar on a weight basis to current doses for beta-carotene and alpha-tocopherol.

Side Effects: There are no known side effects.

(Source: <http://www.beta-glucan-info.com/astaxanthin-questions-answers.htm>)

Research Overview

1. Interferes with proinflammatory substances
2. Blocks nitric oxide enzyme activity
3. Is a powerful antioxidant
4. Astaxanthin bioavailability is increased by some long chain triglycerides
5. Protects beta-cell function in diabetes
6. Reduces glucose toxicity
7. Limits exercise-induced cardiac and skeletal damage in mice
8. Protects against DNA damage from UVA rays
9. Reduces stress-induced lipid peroxidation
10. Helps prevent atherosclerosis
11. Slows growth of H pylori infection
12. Inhibits tumor growth
13. Controls cancer cell proliferation in colon cancer
14. Controls cancer cell proliferation in bladder cancer

Astaxanthin Abstracts (44)

1. Mol Cells. 2003 Aug 31;16(1):97-105.

Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation.

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Astaxanthin, a carotenoid without vitamin A activity, has shown anti-oxidant and anti-inflammatory activities; however, its molecular action and mechanism have not been elucidated. We examined in vitro and in vivo regulatory function of astaxanthin on production of nitric oxide (NO) and prostaglandin E2 (PGE2) as well as expression of inducible NO synthase (iNOS), cyclooxygenase-2, tumor necrosis factor-alpha (TNF-alpha), and interleukin-1beta (IL-1beta). Astaxanthin inhibited the expression or formation production of these proinflammatory mediators and cytokines in both lipopolysaccharide (LPS)-stimulated RAW264.7 cells and primary macrophages. Astaxanthin also suppressed the serum levels of NO, PGE2, TNF-alpha, and IL-1beta in LPS-administrated mice, and inhibited NF-kappaB activation as well as iNOS promoter activity in RAW264.7 cells stimulated with LPS. This compound directly inhibited the intracellular accumulation of reactive oxygen species in LPS-stimulated RAW264.7 cells as well as H2O2-induced NF-kappaB activation and iNOS expression. Moreover, astaxanthin blocked nuclear translocation of NF-kappaB p65 subunit and I(kappa)B(alpha) degradation, which correlated with its inhibitory effect on I(kappa)B kinase (IKK) activity. These results suggest that astaxanthin, probably due to its antioxidant activity, inhibits the production of inflammatory mediators by blocking NF-kappaB activation and as a consequent suppression of IKK activity and I(kappa)B-alpha degradation.

2. Biochem Biophys Res Commun. 2003 Aug 1;307(3):704-12.

Direct superoxide anion scavenging by a disodium disuccinate astaxanthin derivative: Relative efficacy of individual stereoisomers versus the statistical mixture of stereoisomers by electron paramagnetic resonance imaging.

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Carotenoids are a related group of greater than 600 natural compounds, irrespective of geometric- and stereoisomers, with demonstrated antioxidant efficacy. The carotenoids are broadly divided into "carotenes," or non-oxygen substituted hydrocarbon carotenoids, and "xanthophylls," oxygen-substituted carotenoids. The natural compounds are excellent singlet oxygen quenchers as well as lipid peroxidation chain-breakers; this dual antioxidant capacity is generally attributed to the activity of the polyene chain, and increases with the number of conjugated double bonds along the polyene chain length. However, the poor aqueous solubility of most carotenes and the vast majority of xanthophylls limits their use as aqueous-phase singlet oxygen quenchers and direct radical scavengers. A variety of introduction vehicles (e.g., organic solvents, cyclodextrins) have been used to introduce the insoluble carotenoids into aqueous test systems. Hawaii Biotech, Inc. (HBI) successfully synthesized a novel carotenoid derivative, the disodium disuccinate derivative of astaxanthin (3,3(')-dihydroxy-beta,beta-carotene-4,4(')-dione) in all-trans (all-E) form. The novel derivative is a water-dispersible symmetric chiral molecule with two chiral centers, yielding four stereoisomeric forms: 3R,3(')R and 3S,3(')S (enantiomers), and the diastereomeric meso forms (3R,3(')S and 3(')R,3S). The individual stereoisomers were synthesized at high purity (>90% by HPLC) and compared directly for efficacy with the statistical mixture of stereoisomers obtained from the synthesis from the commercial source of astaxanthin (1:2:1 ratio of 3S,3(')S, meso, and 3R,3(')R, respectively). Direct scavenging of superoxide anion was evaluated in a standard in vitro isolated human neutrophil assay by electron paramagnetic resonance (EPR) imaging, employing the spin-trap DEPMPO. Each novel derivative was tested in pure aqueous formulation and in ethanolic formulation shown to completely disaggregate the compounds in solution. In each case, the ethanolic formulation was a more potent scavenging vehicle. No significant differences in scavenging efficiency were noted among the individual stereoisomers and the statistical mixture of stereoisomers, suggesting that the polyene chain alone was responsible for superoxide scavenging. Dose-ranging revealed that the statistical mixture of stereoisomers of the novel derivative, at millimolar (mM) concentrations, could nearly completely eliminate the superoxide anion signal generated in the activated human neutrophil assay. All ethanolic formulations of the novel derivatives exhibited increased scavenging efficiency over equimolar concentrations of non-esterified astaxanthin delivered in a dimethyl sulfoxide (DMSO) vehicle. These novel compounds will likely find utility in applications requiring aqueous delivery of a highly potent direct radical scavenger.

3. Eur J Pharm Sci. 2003 Jul;19(4):299-304.

Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations.

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Astaxanthin is a carotenoid with antioxidant properties, synthesised by plants and algae, and distributed in marine seafood. Astaxanthin is also available as a food supplement, but, like other carotenoids, is a very lipophilic compound and has low oral bioavailability. However, bioavailability can be enhanced in the presence of fat. There is not much information in the literature about the pharmacokinetics of oral astaxanthin in humans. In this open parallel study, healthy male volunteers received a single dose of 40 mg astaxanthin, as lipid based formulations or as a commercially available food supplement, followed by blood sampling for further analysis of plasma concentrations. Pharmacokinetic parameters were calculated to evaluate the extent and rate of absorption from each formulation. The elimination half-life was 15.9±/5.3 h (n=32), and showed a mono-phasic curve. Three lipid based formulations: long-chain triglyceride (palm oil) and polysorbate 80 (formulation A), glycerol mono- and dioleate and polysorbate 80 (formulation B), and glycerol mono- and dioleate, polysorbate 80 and sorbitan monooleate (formulation C), all showed enhanced bioavailability, ranging from 1.7 to 3.7 times that of the reference formulation. The highest bioavailability was observed with formulation B, containing a high content of the hydrophilic synthetic surfactant polysorbate 80.

4. J Med Food. 2003 Spring;6(1):51-6.

Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial.

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A growing body of scientific literature indicates that astaxanthin is a more powerful antioxidant than other carotenoids and vitamin E and may confer numerous health benefits. The purpose of this investigation was to conduct a human safety study with a *Haematococcus pluvialis* algal extract with high levels of astaxanthin. Thirty-five healthy adults age 35-69 years were enrolled in a randomized, double-blind, placebo-controlled trial of 8 weeks' duration. All participants took three gelscaps per day, one at each meal. Nineteen participants received gelscaps with an algal extract in safflower oil, containing 2 mg of astaxanthin each (treatment); 16 participants received gelscaps containing safflower oil only (placebo). Blood pressure and blood chemistry tests, including a comprehensive metabolic panel and cell blood count, were conducted at the beginning of the trial and after 4 and 8 weeks of supplementation. No significant differences were detected between the treatment and the placebo groups after 8 weeks of supplementation with the algal extract in the parameters analyzed, except for serum calcium, total protein, and eosinophils (P <.01). Although the differences in these three parameters were statistically significant, they were very small and are of no clinical importance. These results reveal that 6 mg of astaxanthin per day from a *H. pluvialis* algal extract can be safely consumed by healthy adults.

5. Invest Ophthalmol Vis Sci. 2003 Jun;44(6):2694-701.

Effects of astaxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo.

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PURPOSE: Astaxanthin (AST) is a carotenoid that is found in marine animals and vegetables. Several previous studies have demonstrated that AST exhibits a wide variety of biological activities including antioxidant, antitumor, and anti-*Helicobacter pylori* effects. In this study, attention was focused on the antioxidant effect of AST. The object of the present study was to investigate the efficacy of AST in endotoxin-induced uveitis (EIU) in rats. In addition, the effect of AST on endotoxin-induced nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor (TNF)-alpha production in a mouse macrophage cell line (RAW 264.7) was studied in vitro. **METHODS:** EIU was induced in male Lewis rats by a footpad injection of lipopolysaccharide (LPS). AST or prednisolone was administered intravenously at 30 minutes before, at the same time as, or at 30 minutes after LPS treatment. The number of infiltrating cells and protein concentration in the aqueous humor collected at 24 hours after LPS treatment was determined. RAW 264.7 cells were pretreated with various concentrations of AST for 24 hours and subsequently stimulated with 10 microg/mL of LPS for 24 hours. The levels of PGE2, TNF-alpha, and NO production were determined in vivo and in vitro. **RESULTS:** AST suppressed the development of EIU in a dose-dependent fashion. The anti-inflammatory effect of 100 mg/kg AST was as strong as that of 10 mg/kg prednisolone. AST also decreased production of NO, activity of inducible nitric oxide synthase (NOS), and production of PGE2 and TNF-alpha in RAW264.7 cells in vitro in a dose-dependent manner. **CONCLUSIONS:** This study suggests that AST has a dose-dependent ocular anti-inflammatory effect, by the suppression of NO, PGE2, and TNF-alpha production, through directly blocking NOS enzyme activity.

6. Trends Biotechnol. 2003 May;21(5):210-6.

Haematococcus astaxanthin: applications for human health and nutrition.

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The carotenoid pigment astaxanthin has important applications in the nutraceutical, cosmetics, food and feed industries. Haematococcus pluvialis is the richest source of natural astaxanthin and is now cultivated at industrial scale. Astaxanthin is a strong coloring agent and a potent antioxidant - its strong antioxidant activity points to its potential to target several health conditions. This article covers the antioxidant, UV-light protection, anti-inflammatory and other properties of astaxanthin and its possible role in many human health problems. The research reviewed supports the assumption that protecting body tissues from oxidative damage with daily ingestion of natural astaxanthin might be a practical and beneficial strategy in health management.

7. Redox Rep. 2002;7(5):290-3.

Astaxanthin protects beta-cells against glucose toxicity in diabetic db/db mice.

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Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic beta-cells and various forms of tissue damage in patients with diabetes mellitus. Astaxanthin, a carotenoid of marine microalgae, is reported as a strong anti-oxidant inhibiting lipid peroxidation and scavenging reactive oxygen species. The aim of the present study was to examine whether astaxanthin can elicit beneficial effects on the progressive destruction of pancreatic beta-cells in db/db mice--a well-known obese model of type 2 diabetes. We used diabetic C57BL/KsJ-db/db mice and db/m for the control. Astaxanthin treatment was started at 6 weeks of age and its effects were evaluated at 10, 14, and 18 weeks of age by non-fasting blood glucose levels, intraperitoneal glucose tolerance test including insulin secretion, and beta-cell histology. The non-fasting blood glucose level in 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 intraperitoneal glucose tolerance test, was preserved in the astaxanthin-treated group. Histology of the pancreas revealed no significant differences in the beta-cell mass between astaxanthin-treated and -untreated db/db mice. In conclusion, these results indicate that astaxanthin can exert beneficial effects in diabetes, with preservation of beta-cell function. This finding suggests that anti-oxidants may be potentially useful for reducing glucose toxicity.

8. J Pharm Sci. 2003 Apr;92(4):922-6.

Improved aqueous solubility of crystalline astaxanthin (3,3'-dihydroxy-beta, beta-carotene-4,4'-dione) by Captisol (sulfobutyl ether beta-cyclodextrin).

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Carotenoids are the most widely distributed natural pigments, with over 600 individual compounds identified and characterized from natural sources. A few are commercially important molecules, having found utility as additions to animal feed in the aquaculture, poultry, and swine feed industries. The majority are lipophilic molecules with near zero inherent aqueous solubility. Many different methods have been developed to make the carotenoids "water dispersible," as true water solubility has not been described. Astaxanthin (3,3'-dihydroxy-beta, beta-carotene-4,4'-dione) is a commercially important oxygenated carotenoid that has gained wide acceptance as a feed additive in the \$50 billion salmon and trout aquaculture industry. Recently, interest in the human health applications of astaxanthin has increased, with astaxanthin receiving approval as a dietary supplement in several countries, including the United States. Moving astaxanthin into a pharmaceutical application will require a chemical delivery system that overcomes the problems with parenteral administration of a highly lipophilic, low molecular weight compound. In the current study, the ability of sulfobutyl ether beta-cyclodextrin (sodium), as the Captisol(R) brand, to increase the aqueous water solubility of crystalline astaxanthin was evaluated. Complexation of crystalline astaxanthin with Captisol increased the apparent water solubility of crystalline astaxanthin approximately 71-fold, to a concentration in the 2 microg/mL range. It is unlikely that this increase in solubility will result in a pharmaceutically acceptable chemical delivery system for humans. However, the increased aqueous solubility of crystalline astaxanthin to the range achieved in the current study will likely find utility in the introduction of crystalline astaxanthin into mammalian cell culture systems that have previously been dependent upon liposomes, or toxic organic solvents,

9. Antioxid Redox Signal. 2003 Feb;5(1):139-44.

Astaxanthin limits exercise-induced skeletal and cardiac muscle damage in mice.

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Dietary antioxidants may attenuate oxidative damage from strenuous exercise in various tissues. Beneficial effects of the antioxidant astaxanthin have been demonstrated in vitro, but not yet in vivo. We investigated the effect of dietary supplementation with astaxanthin on oxidative damage induced by strenuous exercise in mouse gastrocnemius and heart. C57BL/6 mice (7 weeks old) were divided into groups: rested control, intense exercise, and exercise with astaxanthin supplementation. After 3 weeks of exercise acclimation, both exercise groups ran on a treadmill at 28 m/min until exhaustion. Exercise-increased 4-hydroxy-2-nonenal-modified protein and 8-hydroxy-2'-deoxyguanosine in gastrocnemius and heart were blunted in the astaxanthin group. Increases in plasma creatine kinase activity, and in myeloperoxidase activity in gastrocnemius and heart, also were lessened by astaxanthin. Astaxanthin showed accumulation in gastrocnemius and heart from the 3 week supplementation. Astaxanthin can attenuate exercise-induced damage in mouse skeletal muscle and heart, including an associated neutrophil infiltration that induces further damage.

10. Comp Biochem Physiol C Toxicol Pharmacol. 2002 Nov;133(3):443-51.

Astaxanthin and canthaxanthin do not induce liver or kidney xenobiotic-metabolizing enzymes in rainbow trout (*Oncorhynchus mykiss* Walbaum).

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This study was designed to assess the effects of dietary carotenoid supplementation on liver and kidney xenobiotic-metabolizing enzymes in the rainbow trout. Twelve rainbow trout (mean weight 266±10 g) were assigned to each of three replicate tanks for each of four dietary treatments; astaxanthin, canthaxanthin, negative control and positive control using beta-naphthoflavone, at a target dietary inclusion of 100 mg kg⁻¹ for each additive. Fish were fed for 3 weeks at a level of 1.2% body wt. day⁻¹. Serum carotenoid levels were used as indicators of exposure and were not significantly different ($P>0.05$) between carotenoid-fed trout. Livers and kidney were frozen separately in liquid N₂ by immersion and microsomal fractions from pooled samples (n=3) assayed for xenobiotic-metabolizing enzyme (cytochrome P450 monooxygenase) activities including ethoxyresorufin O-deethylase; methoxyresorufin O-demethylase; pentoxyresorufin O-dealkylase; benzoxyresorufin O-dearylase; and the conjugating enzymes glucuronosyl transferase; and glutathione-s-transferase. Results revealed that carotenoid treatment did not significantly ($P>0.05$) induce any enzyme system examined. Results are discussed in the context of metabolism of absorbed carotenoids.

11. J Dermatol Sci. 2002 Oct;30(1):73-84.

Modulatory effects of an algal extract containing astaxanthin on UVA-irradiated cells in culture.

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UV radiation from sunlight is the most potent environmental risk factor in skin cancer pathogenesis. In the present study the ability of an algal extract to protect against UVA-induced DNA alterations was examined in human skin fibroblasts (1BR-3), human melanocytes (HEMAc) and human intestinal CaCo-2 cells. The protective effects of the proprietary algal extract, which contained a high level of the carotenoid astaxanthin, were compared with synthetic astaxanthin. DNA damage was assessed using the single cell gel electrophoresis or comet assay. In 1BR-3 cells, synthetic astaxanthin prevented UVA-induced DNA damage at all concentrations (10 nM, 100 nM, 10 microM) tested. In addition, the synthetic carotenoid also prevented DNA damage in both the HEMAc and CaCo-2 cells. The algal extract displayed protection against UVA-induced DNA damage when the equivalent of 10 microM astaxanthin was added to all three-cell types, however, at the lower concentrations (10 and 100 nM) no significant protection was evident. There was a 4.6-fold increase in astaxanthin content of CaCo-2 cells exposed to the synthetic compound and a 2.5-fold increase in cells exposed to algal extract. In 1BR-3 cells, exposure to UVA for 2 h resulted in a significant induction

of cellular superoxide dismutase (SOD) activity, coupled with a marked decrease in cellular glutathione (GSH) content. However pre-incubation (18 h) with 10 microM of either the synthetic astaxanthin or the algal extract prevented UVA-induced alterations in SOD activity and GSH content. Similarly, in CaCo-2 cells a significant depletion of GSH was observed following UVA-irradiation which was prevented by simultaneously incubating with 10 microM of either synthetic astaxanthin or the algal extract. SOD activity was unchanged following UVA exposure in the intestinal cell line. This work suggests a role for the algal extract as a potentially beneficial antioxidant.

12. Life Sci. 2002 Apr 21;70(21):2509-20.

Contribution of the antioxidative property of astaxanthin to its protective effect on the promotion of cancer metastasis in mice treated with restraint stress.

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We investigated the effects of astaxanthin on the antitumor effector activity of natural killer (NK) cells suppressed by stress in mice in order to define the immunological significance of astaxanthin (ASX) when combined with restraint stress treatment. When the mice were treated with restraint stress alone, the total number of spleen cells, and the level NK cell activity per spleen were reduced to a nadir on day 3. The stress also caused a significant increase in the lipid peroxidation of liver tissue. ASX (100 mg/kg/day, p.o., 4 days) improved the immunological dysfunction induced by restraint stress. On the other hand, metastatic nodules were observed in the livers of syngenic DBA/2 mice on day 12 after inoculation of P815 mastocytoma cells. Hepatic metastasis was promoted further by restraint stress when applied on day 3 before the inoculation of P815. Daily oral administration of ASX (1 mg/kg/day, p.o., 14 days) markedly attenuated the promotion of hepatic metastasis induced by restraint stress. These results suggested that astaxanthin improves antitumor immune responses by inhibiting of lipid peroxidation induced by stress.

13. Arch Toxicol. 2002 Jan;75(11-12):665-75.

Metabolism and CYP-inducer properties of astaxanthin in man and primary human hepatocytes.

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Previous investigations in the rat have shown that the non-provitamin A carotenoid astaxanthin is metabolized into 3-hydroxy-4-oxo-beta-ionone and 3-hydroxy-4-oxo-7,8-dihydro-beta-ionone and, in addition, is a potent CYP1A gene inducer. Here we investigated the metabolism of this compound as well as its capacity to induce CYP genes in primary cultures of human hepatocytes. Free metabolites of ¹⁴C-astaxanthin produced in this cellular model were purified by high pressure liquid chromatography (HPLC) and identified by gas chromatography-mass spectrometry (GC-MS) analyses as 3-hydroxy-4-oxo-beta-ionol and 3-hydroxy-4-oxo-beta-ionone. In addition, deconjugation of polar compounds by glucuronidase and further analyses with HPLC and GC-MS revealed four radiolabeled metabolites including: 3-hydroxy-4-oxo-beta-ionol, 3-hydroxy-4-oxo-beta-ionone, and their reduced forms, 3-hydroxy-4-oxo-7, 8-dihydro-beta-ionol and 3-hydroxy-4-oxo-7,8-dihydro-beta-ionone. The same four metabolites were identified in human plasma from two volunteers who had orally taken 100 mg astaxanthin 24 h before blood collection. In cultured hepatocytes, astaxanthin was a significant inducer of the major cytochrome P450 enzyme, CYP3A4 as well as of CYP2B6, but not of other CYPs, including those from CYP1A and CYP2C families. The lack of autoinduction of astaxanthin metabolism in human hepatocytes suggests that neither CYP3A4 nor CYP2B6 contribute to the formation of metabolites. We conclude that metabolism of astaxanthin and its CYP-inducing capacity are different in humans and in rats. The novel methodology used in our studies could be extended to evaluating the role of metabolites of more important carotenoids such as beta-carotene in differentiation and carcinogenicity.

14. J Reprod Fertil Suppl. 2001;57:331-4.

Effect of supplementation with the antioxidant astaxanthin on reproduction, pre-weaning growth performance of kits and daily milk intake in mink.

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The study comprised two parts. Firstly, the effects of dietary supplementation with an algal meal (Novasta) with a high astaxanthin content on ovulation rate (number of corpora lutea, implantation rate, number, mass and length of fetuses) of breeding female mink were evaluated. Secondly, reproductive outcome (number of live and stillborn kits), kit growth rate and milk intake were studied. Both studies were performed on standard brown female mink (n = 20; control (n = 10) and experimental (n = 10)) housed under conventional farm conditions. Experimental animals were supplied with 5.35 mg astaxanthin per day (0.25 g algal meal (Novasta)). The numbers of corpora lutea, implantation sites and fetuses appeared to be higher in the group that was given astaxanthin but the effect was not significant. The differences between treated and control mink were 1.4 (corpora lutea), 0.9 (implantation sites) and 1.2 (litter size). The percentage of stillborn kits was reduced by 6.3 (P < 0.005). The milk intake as measured by use of the isotopic water dilution technique was not affected by treatment group. Milk intake increased from about 19 g in week 1 of lactation to about 30 g per kit per day in week 4 of lactation. Kit weight gain was not affected by the experimental treatment.

15. *Biochem Biophys Res Commun.* 2001 Oct 19;288(1):225-32.

Astaxanthin and peridinin inhibit oxidative damage in Fe(2+)-loaded liposomes: scavenging oxyradicals or changing membrane permeability?

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Astaxanthin and peridinin, two typical carotenoids of marine microalgae, and lycopene were incorporated in phosphatidylcholine multilamellar liposomes and tested as inhibitors of lipid oxidation. Contrarily to peridinin results, astaxanthin strongly reduced lipid damage when the lipoperoxidation promoters-H₂O₂, tert-butyl hydroperoxide (t-ButOOH) or ascorbate-and Fe(2+):EDTA were added simultaneously to the liposomes. In order to check if the antioxidant activity of carotenoids was also related to their effect on membrane permeability, the peroxidation processes were initiated by adding the promoters to Fe(2+)-loaded liposomes (encapsulated in the inner aqueous solution). Despite that the rigidifying effect of carotenoids in membranes was not directly measured here, peridinin probably has decreased membrane permeability to initiators (t-ButOOH > ascorbate > H₂O₂) since its incorporation limited oxidative damage on iron-liposomes. On the other hand, the antioxidant activity of astaxanthin in iron-containing vesicles might be derived from its known rigidifying effect and the inherent scavenging ability. Copyright 2001 Academic Press.

16. *J Atheroscler Thromb.* 2000;7(4):216-22.

Inhibition of low-density lipoprotein oxidation by astaxanthin.

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Marine animals produce astaxanthin which is a carotenoid and antioxidant. In this study we determined the in vitro and ex vivo effects of astaxanthin on LDL oxidation. The oxidation of LDL was measured in a 1 ml reaction system consisting of increasing concentrations of astaxanthin (12.5, 25.0, 50.0 microg/ml), 400 microM V-70 (2, 2'-azobis(4-methoxy-2, 4-dimethylvaleronitrile)), and LDL (70 microg/ml protein). Astaxanthin dose, dependently significantly prolonged the oxidation lag time (31.5, 45.4, 65.0 min) compared with the control (19.9 min). For the ex vivo study 24 volunteers (mean age 28.2 [SD 7.8] years) consumed astaxanthin at doses of 1.8, 3.6, 14.4 and 21.6 mg per day for 14 days. No other changes were made in the diet. Fasting venous blood samples were taken at days 0, +14. LDL lag time was longer (5.0, 26.2, 42.3 and 30.7% respectively) compared with day 0 after consuming astaxanthin at doses of 1.8, 3.6, 14.4 and 21.6 mg for 14 days compared with day 0, but there was no difference in oxidation of LDL between day 0 (lag time 59.9+/-7.2 min) and day 14 (57.2+/-6.0 min) in the control group. Our results provide evidence that consumption of marine animals producing astaxanthin inhibits LDL oxidation and possibly therefore contributes to the prevention of atherosclerosis.

17. *Methods Find Exp Clin Pharmacol.* 2001 Mar;23(2):79-84.

Effect of astaxanthin on the hepatotoxicity, lipid peroxidation and antioxidative enzymes in the liver of CCl₄-treated rats.

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Astaxanthin is one of many carotenoids present in marine animals, vegetables and fruits. Since carotenoids are known to have antioxidant properties, we tested to determine if astaxanthin could have protective effects in the CCl₄-treated rat liver by activating

the antioxidant system. Astaxanthin blocked the increase of glutamate-oxalacetate transaminase (GOT) and glutamate-pyruvate transaminase (GTP) activity and thiobarbituric acid reactive substances (TBARS) in response to carbon tetrachloride (CCl₄), while causing an increase in glutathione (GSH) levels and superoxide dismutase (SOD) activities in the CCl₄-treated rat liver. These results suggest that astaxanthin protects liver damage induced by CCl₄ by inhibiting lipid peroxidation and stimulating the cellular antioxidant system.

18. *Biochim Biophys Acta*. 2001 Jun 6;1512(2):251-8.

Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin.

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The effects of the carotenoids beta-carotene and astaxanthin on the peroxidation of liposomes induced by ADP and Fe(2+) were examined. Both compounds inhibited production of lipid peroxides, astaxanthin being about 2-fold more effective than beta-carotene. The difference in the modes of destruction of the conjugated polyene chain between beta-carotene and astaxanthin suggested that the conjugated polyene moiety and terminal ring moieties of the more potent astaxanthin trapped radicals in the membrane and both at the membrane surface and in the membrane, respectively, whereas only the conjugated polyene chain of beta-carotene was responsible for radical trapping near the membrane surface and in the interior of the membrane. The efficient antioxidant activity of astaxanthin is suggested to be due to the unique structure of the terminal ring moiety.

19. 0955-2863. 2000 Oct;11(10):482-490.

Plasma appearance and distribution of astaxanthin E/Z and R/S isomers in plasma lipoproteins of men after single dose administration of astaxanthin(1).

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Appearance, pharmacokinetics, and distribution of astaxanthin E/Z and R/S isomers in plasma and lipoprotein fractions were studied in 3 middle-aged male volunteers (37-43 years) after ingestion of a single meal containing a 100 mg dose of astaxanthin. The astaxanthin source consisted of 74% all-E-, 9% 9Z-, 17% 13Z-astaxanthin (3R,3'R-, 3R,3'S; meso-, and 3S,3'S-astaxanthin in a 1:2:1 ratio). The plasma astaxanthin concentration--time curves were measured during 72 hr. Maximum levels of astaxanthin (1.3 +/- 0.1 mg/L) were reached 6.7 +/- 1.2 hr after administration, and the plasma astaxanthin elimination half-life was 21 +/- 11 hr. 13Z-Astaxanthin accumulated selectively, whereas the 3 and 3'R/S astaxanthin distribution was similar to that of the experimental meal. 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. The astaxanthin isomer distribution in plasma, VLDL/CM, LDL, and HDL was not affected by time. The results indicate that a selective process increases the relative proportion of astaxanthin Z-isomers compared to the all-E-astaxanthin during blood uptake and that astaxanthin E/Z isomers have similar pharmacokinetics.

20. *Antimicrob Agents Chemother*. 2000 Sep;44(9):2452-7.

Astaxanthin-rich algal meal and vitamin C inhibit *Helicobacter pylori* infection in BALB/cA mice.

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Helicobacter pylori infection in humans is associated with chronic type B gastritis, peptic ulcer disease, and gastric carcinoma. A high intake of carotenoids and vitamin C has been proposed to prevent development of gastric malignancies. The aim of this study was to explore if the microalga *Haematococcus pluvialis* rich in the carotenoid astaxanthin and vitamin C can inhibit experimental *H. pylori* infection in a BALB/cA mouse model. Six-week-old BALB/cA mice were infected with the mouse-passaged *H. pylori* strain 119/95. At 2 weeks postinoculation mice were treated orally once daily for 10 days (i) with different doses of algal meal rich in astaxanthin (0.4, 2, and 4 g/kg of body weight, with the astaxanthin content at 10, 50, and 100 mg/kg, respectively), (ii) with a control meal (algal meal without astaxanthin, 4 g/kg), or (iii) with vitamin C (400 mg/kg). Five mice from each group were sacrificed 1 day after the cessation of treatment, and the other five animals were sacrificed 10 days after the cessation of treatment. Culture of *H. pylori* and determination of the inflammation score of the gastric mucosae were used to determine the outcome of the

treatment. Mice treated 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 1 day and 10 days after the cessation of treatment. Lipid peroxidation was significantly decreased in mice treated with the astaxanthin-rich algal meal and vitamin C compared with that of animals not treated or treated with the control meal. Both astaxanthin-rich algal meal and vitamin C showed an inhibitory effect on *H. pylori* growth in vitro. In conclusion, antioxidants may be a new strategy for treating *H. pylori* infection in humans.

21. *J Nutr.* 2000 Jul;130(7):1800-8.

Depletion of alpha-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism.

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Duplicate groups of Atlantic salmon post-smolts were fed four purified diets supplemented with both vitamin E and the carotenoid astaxanthin (Ax) (+E, +Ax), or supplemented with either vitamin E or Ax (-E, +Ax and +E, -Ax) or deficient in both vitamin E and Ax (-E, -Ax) for 22 wk. There were no effects of diet on growth rate, but an extensive lipid liver degenerative lesion was observed in 15% of fish fed diets deficient in vitamin E. Tissue vitamin E concentrations varied in accordance with dietary vitamin E in liver, muscle, heart, plasma, brain and eye; levels were reduced to approximately 3% in liver but only to 40% in eye of fish fed diets deficient in vitamin E compared with those fed diets supplemented with vitamin E. An interactive sparing of Ax supplementation on tissue vitamin E concentration was observed, but only in brain. Dietary deficiency of both vitamin E and Ax significantly increased the recovery of desaturated and elongated products of both [1-(14)C] 18:3(n-3) and [1-(14)C] 20:5(n-3) in isolated hepatocytes, suggesting that conversion of fatty acids to their long-chain highly unsaturated products can be stimulated by a deficiency of lipid-soluble antioxidants. The antioxidant synergism of vitamin E and Ax was supported by their ability to reduce malondialdehyde formation in an in vitro stimulation of microsomal lipid peroxidation and to reduce plasma levels of 8-isoprostane. The results of this study suggest that both vitamin E and the carotenoid Ax have antioxidant functions in Atlantic salmon.

22. *Nutr Cancer.* 2000;36(1):59-65.

Antitumor activity of astaxanthin and its mode of action.

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Astaxanthin, a carotenoid without vitamin A activity, may exert antitumor activity through the enhancement of immune responses. Here, we determined the effects of dietary astaxanthin on tumor growth and tumor immunity against transplantable methylcholanthrene-induced fibrosarcoma (Meth-A tumor) cells. These tumor cells express a tumor antigen that induces T cell-mediated immune responses in syngenic mice. BALB/c mice were fed astaxanthin (0.02%, 40 micrograms/kg body wt/day in a beadlet form) mixed in a chemically defined diet starting zero, one, and three weeks before subcutaneous inoculation with tumor cells (3×10^5) cells, 2 times the minimal tumorigenic dose). Three weeks after inoculation, tumor size and weight were determined. We also determined cytotoxic T lymphocyte (CTL) activity and interferon-gamma (IFN-gamma) production by tumor-draining lymph node (TDLN) and spleen cells by restimulating cells with Meth-A tumor cells in culture. 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 antitumor activity was paralleled with higher CTL activity and IFN-gamma production by TDLN and spleen cells in the astaxanthin-fed mice. CTL activity by TDLN 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 IFN-gamma production by spleen cells. Total serum astaxanthin concentrations were approximately 1.2 $\mu\text{mol/l}$ when mice were fed astaxanthin (0.02%) for four weeks and appeared to increase in correlation with the length of astaxanthin supplementation. Our results indicate that dietary astaxanthin suppressed Meth-A tumor cell growth and stimulated immunity against Meth-A tumor antigen.

23. *J Agric Food Chem.* 2000 Apr;48(4):1150-4.

Antioxidant activities of astaxanthin and related carotenoids.

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The antioxidant activities of astaxanthin and related carotenoids have been measured by employing a newly developed fluorometric assay. This assay is based on 4,4-difluoro-3,5-bis(4-phenyl-1, 3-butadienyl)-4-bora-3a,4a-diaza-s-indacene (BODIPY 665/676) as an indicator; 2,2'-azobis-2,4-dimethylvaleronitrile (AMVN) as a peroxy radical generator; and 6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid (Trolox) as a calibrator in an organic and liposomal media. By employing this assay, three categories of carotenoids were examined: namely, the hydrocarbon carotenoids lycopene, alpha-carotene, and beta-carotene; the hydroxy carotenoid lutein; and the alpha-hydroxy-ketocarotenoid astaxanthin. The relative peroxy radical scavenging activities of Trolox, astaxanthin, alpha-tocopherol, lycopene, beta-carotene, lutein, and alpha-carotene in octane/butyronitrile (9:1, v/v) were determined to be 1.0, 1.0, 1.3, 0.5, 0.4, 0.3, and 0.2, respectively. In dioleoylphosphatidyl choline (DOPC) liposomal suspension in Tri-HCl buffer (pH 7.4 at 40 degrees C), the relative reactivities of astaxanthin, beta-carotene, alpha-tocopherol, and lutein were found to be 1.00, 0.9, 0.6, and 0.6, respectively. When BODIPY 665/676 was replaced by 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a, 4a-diaza-s-indacene-3-undecanoic acid (BODIPY 581/591 C(11)) as an indicator, astaxanthin showed the highest antioxidant activity toward peroxy radicals. The relative reactivities of Trolox, astaxanthin, alpha-tocopherol, alpha-carotene, lutein, beta-carotene, and lycopene were determined to be 1.0, 1.3, 0.9, 0.5, 0.4, 0.2, and 0.4, respectively.

24. *Anticancer Res.* 1999 Nov-Dec;19(6B):5223-7.

Dietary beta-carotene and astaxanthin but not canthaxanthin stimulate splenocyte function in mice.

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The in vivo modulatory effect of beta-carotene, astaxanthin and canthaxanthin on lymphocyte function was investigated. Female BALB/c mice (8 wk old) were fed a basal diet containing 0, 0.1% or 0.4% beta-carotene, astaxanthin or canthaxanthin for 0, 2 or 4 wk (n = 8/diet/period). Splenic lymphocytes were isolated and mitogen-stimulated proliferation, IL-2 production and lymphocyte cytotoxicity were assessed. Body weight and feed intake were not different among dietary treatments. Plasma carotenoids were undetectable in unsupplemented mice but concentrations of the respective carotenoids were elevated in mice fed 0.1 or 0.4% beta-carotene (0.22 and 0.39 $\mu\text{mol/L}$), astaxanthin (16.4 and 50.2 $\mu\text{mol/L}$) and canthaxanthin (5.00 and 7.02 $\mu\text{mol/L}$) respectively. Mice fed both dietary levels of beta-carotene and astaxanthin had enhanced phytohemagglutinin-induced lymphoblastogenesis compared to unsupplemented mice ($P < 0.03$). No treatment difference was detected with concanavalin A- or lipopolysaccharide-induced lympho-proliferation nor with IL-2 production ($P < 0.05$). Astaxanthin (0.1%) also enhanced lymphocyte cytotoxic activity ($P < 0.08$). In contrast, canthaxanthin did not significantly influence any of the lymphocyte functions measured. Results indicate that beta-carotene and astaxanthin but not canthaxanthin exert enhanced splenic lymphocyte function in mice.

25. *Immunol Lett.* 1999 Dec 1;70(3):185-9.

Treatment of *H. pylori* infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes.

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Helicobacter pylori is a gram-negative bacterium affecting about half of the world population, causing chronic gastritis type B dominated by activated phagocytes. In some patients the disease evolves into gastric ulcer, duodenal ulcer, gastric cancer or MALT lymphoma. The pathogenesis is in part caused by the immunological response. In mouse models and in human disease, the mucosal immune response is characterized by activated phagocytes. Mucosal T-lymphocytes are producing IFN-gamma thus increasing mucosal inflammation and mucosal damage. A low dietary intake of antioxidants such as carotenoids and vitamin C may be an important factor for acquisition of *H. pylori* by humans. Dietary antioxidants may also affect both acquisition of the infection and the bacterial load of *H. pylori* infected mice. Antioxidants, including carotenoids, have anti-inflammatory effects. The aim of the present study was to investigate whether dietary antioxidant induced modulation of *H. pylori* in mice affected the cytokines produced by *H. pylori* specific T-cells. We found that treatment of *H. pylori* infected mice with an algal cell extract containing the antioxidant astaxanthin reduces bacterial load and gastric inflammation. These changes are associated with a shift of the T-lymphocyte response from a predominant Th1-response dominated by IFN-gamma to a Th1/Th2-response with IFN-gamma and IL-4. To our knowledge, a switch from a Th1-response to a mixed Th1/Th2-response during an ongoing infection has not been reported previously.

26. *Anticancer Res.* 1999 May-Jun;19(3A):1849-53.

A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo.

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The anticancer activities of beta-carotene, astaxanthin and canthaxanthin against the growth of mammary tumors were studied in female eight-wk-old BALB/c mice. The mice were fed a synthetic diet containing 0, 0.1 or 0.4% beta-carotene, astaxanthin or canthaxanthin. After 3 weeks, all mice were inoculated with 1×10^6 WAZ-2T tumor cells into the mammary fat pad. All animals were killed on 45 d after inoculation with the tumor cells. No carotenoids were detectable in the plasma or tumor tissues of unsupplemented mice. Concentrations of plasma astaxanthin (20 to 28 $\mu\text{mol/L}$) were greater ($P < 0.05$) than that of beta-carotene (0.1 to 0.2 $\mu\text{mol/L}$) and canthaxanthin (3 to 6 mmol/L). However, in tumor tissues, the concentration of canthaxanthin (4.9 to 6.0 nmol/g) was higher than that of beta-carotene (0.2 to 0.5 nmol/g) and astaxanthin (1.2 to 2.7 nmol/g). In general, all three carotenoids decreased mammary tumor volume. Mammary tumor growth inhibition by astaxanthin was dose-dependent and was higher than that of canthaxanthin and beta-carotene. Mice fed 0.4% beta-carotene or canthaxanthin did not show further increases in tumor growth inhibition compared to those fed 0.1% of each carotenoid. Lipid peroxidation activity in tumors was lower ($P < 0.05$) in mice fed 0.4% astaxanthin, but not in those fed beta-carotene and canthaxanthin. Therefore, beta-carotene, canthaxanthin and especially astaxanthin inhibit the growth of mammary tumors in mice; their anti-tumor activity is also influenced by the supplemental dose.

27. *Nutr Cancer*. 1999;33(2):206-12.

Dietary lutein but not astaxanthin or beta-carotene increases pim-1 gene expression in murine lymphocytes.

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This study investigates the effect of dietary carotenoids on pim-1 gene expression in mouse splenocytes. Female BALB/c mice were fed 0%, 0.02%, or 0.4% astaxanthin, beta-carotene, and lutein for two weeks. Plasma and liver were obtained for the analysis of carotenoids. Splenocytes were isolated and cultured in the presence of concanavalin A, and the level of pim-1 mRNA was determined by Northern blot analysis. None of the carotenoids were detectable in the plasma and liver of unsupplemented mice. In plasma the concentration of astaxanthin (4.9-54.7 $\mu\text{mol/l}$) was dramatically higher than that of lutein (1.4-2.0 $\mu\text{mol/l}$) and beta-carotene (0.1-0.7 $\mu\text{mol/l}$). Carotenoid uptake by the spleen but not the liver reflected that observed in plasma. In mice fed 0.4% of each carotenoid, the absolute concentration of the carotenoid in the liver was highest for astaxanthin (24 nmol/g) followed by beta-carotene (7.5 nmol/g) and lutein (1.58 nmol/g). Mice fed lutein showed a dose-related increase in pim-1 mRNA expression. The steady-state level of pim-1 mRNA in mice fed 0.4% lutein was sixfold higher than in mice fed 0.02% lutein. In contrast, dietary astaxanthin and beta-carotene did not affect pim-1 expression. Therefore, an increase in pim-1 mRNA was observed in splenocytes stimulated with concanavalin A in lutein-fed mice. This appears to be a unique effect of lutein and may be associated with its antitumor activity observed in vivo.

28. *Drug Metab Dispos*. 1999 Apr;27(4):456-62.

Characterization of metabolites of astaxanthin in primary cultures of rat hepatocytes.

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The metabolism of the nonprovitamin A carotenoid astaxanthin was investigated in primary cultures of rat hepatocytes. In a time course study based on HPLC and gas chromatography-mass spectrometry analyses, one main metabolite, (rac)-3-hydroxy-4-oxo-beta-ionone, was found. This metabolite was conjugated mainly into glucuronides, as demonstrated by glucuronidase treatment of the conjugates under sulfatase-inhibiting conditions. Within 24 h more than 50% astaxanthin was metabolized and conjugated. Deconjugation of the polar conjugates with glucuronidase and analyses with HPLC and gas chromatography-mass spectrometry identified two metabolites, (rac)-3-hydroxy-4-oxo-beta-ionone and its reduced form (rac)-3-hydroxy-4-oxo-7,8-dihydro-beta-ionone, indicating that the former was reduced in the conjugated form. We confirmed that the ketocarotenoid astaxanthin induces xenobiotic-metabolizing enzymes in rat liver 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 indicated that the cytochrome P-450 enzymes were not involved in the metabolism of astaxanthin in rat hepatocytes. We conclude that astaxanthin was metabolized in primary cultures of rat hepatocytes into (rac)-3-hydroxy-4-oxo-beta-ionone and its reduced form (rac)-3-hydroxy-4-oxo-7,8-dihydro-beta-ionone independent of the xenobiotic-metabolizing enzymes induced by astaxanthin.

Modulation of UVA light-induced oxidative stress by beta-carotene, lutein and astaxanthin in cultured fibroblasts.

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The ability of beta-carotene, lutein or astaxanthin to protect against UVA-induced oxidative stress in rat kidney fibroblasts (NRK) was assessed. Activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), and changes in thiobarbituric acid reactive substances (TBARS) were measured as indices of oxidative stress. Exposure to UVA light at a dose intensity of 5.6 mW/cm² for 4 h resulted in a significant decrease in CAT and SOD activities and a significant increase in TBARS. No cytotoxicity, as indicated by lactate dehydrogenase (LDH) release, was observed. beta-Carotene (1 microM), lutein (1 microM) and astaxanthin (10 nM) protect against UVA light-induced oxidative stress in vitro with astaxanthin exhibiting superior protective properties.

30. Z Naturforsch [C]. 1998 Jan-Feb;53(1-2):93-100.

Does astaxanthin protect Haematococcus against light damage?

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The photoprotective function of the ketocarotenoid astaxanthin in Haematococcus was questioned. When exposed to high irradiance and/or nutritional stress, green Haematococcus cells turned red due to accumulation of an immense quantity of the red pigment astaxanthin. Our results demonstrate that: 1) The addition of diphenylamine, an inhibitor of astaxanthin biosynthesis, causes cell death under high light intensity; 2) Red cells are susceptible to high light stress to the same extent or even higher than green ones upon exposure to a very high light intensity (4000 μmol photon m⁻²s⁻¹); 3) Addition of 1O₂ generators (methylene blue, rose bengal) under noninductive conditions (low light of 100 μmol photon m⁻²s⁻¹) induced astaxanthin accumulation. This can be reversed by an exogenous 1O₂ quencher (histidine); 4) Histidine can prevent the accumulation of astaxanthin induced by phosphate starvation. We suggest that: 1) Astaxanthin is the result of the photoprotection process rather than the protective; 2) 1O₂ is involved indirectly in astaxanthin accumulation process.

31. J Nutr Sci Vitaminol (Tokyo). 1997 Jun;43(3):345-55.

Inhibition of beta-carotene and astaxanthin of NADPH-dependent microsomal phospholipid peroxidation.

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To evaluate the antioxidant effects of beta-carotene and astaxanthin, rat liver microsomes were exposed to a mixture of chelated iron (Fe³⁺/ADP) and NADPH. The carotenoids (190 pmol/mg protein) were incorporated into some of these microsomal membranes, and phospholipid hydroperoxides (PLOOH), thiobarbituric acid reactive substances (TBARS) and endogenous alpha-tocopherol content were measured over time after the initiation of oxidant stress. In control microsomes, oxidant stress led to accumulation of 1,865 (+/- 371) pmol PLOOH/mg protein during the initial 10-min peroxidation reaction, followed by a more gradual decrease during the subsequent 20-min of reaction. PLOOH accumulation during the initial 10-min reaction period was reduced to 588 (+/- 169) pmol/mg protein with beta-carotene present and 800 (+/- 288) pmol/mg protein with astaxanthin present. During the following 20-min of incubation, PLOOH levels declined in the carotenoid-supplemented microsomes but continued to increase at a slower rate in control preparations. TBARS did not show such large accumulation as observed in PLOOH during the initial 10-min incubation in any microsomal sample. The presence of carotenoids in the microsomal membrane partially inhibited the loss of alpha-tocopherol, especially during the later phase of oxidant stress. When lipid peroxidation is generated by membrane-bound cytochrome P-450, the specific measurement of PLOOH clearly demonstrates that the presence of carotenoids provides antioxidant protection.

32. Xenobiotica. 1996 Jan;26(1):49-63.

Effects of canthaxanthin, astaxanthin, lycopene and lutein on liver xenobiotic-metabolizing enzymes in the rat.

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1. The catalytic activities of several phase I and II xenobiotic-metabolizing enzymes and the immunochemical detection of P4501A and 2B have been investigated in liver microsomes and cytosol of male rats fed for 15 days with diets containing canthaxanthin, astaxanthin, lycopene or lutein (as lutein esters) (300 mg/kg diet) and in rats fed increasing levels (10, 30, 100 and 300 ppm) of canthaxanthin or astaxanthin in the diet. 2. Canthaxanthin increased the liver content of P450, the activities of NADH- and NADPH-cytochrome c reductase, and produced a substantial increase of some P450-dependent activities, especially ethoxyresorufin O-deethylase (EROD) (x 139) and methoxyresorufin O-demethylase (MROD) (x 26). Canthaxanthin also increased pentoxy-(PROD) and benzoxyresorufin O-dealkylases (BROD), but did not affect. NADPH-cytochrome c reductase and erythromycin N-demethylase (ERDM) activities and decreased nitrosodimethylamine N-demethylase (NDMAD) activity. Phase II p-nitrophenol UDP-glucuronosyl transferase (4NP-UGT) and quinone reductase (QR) activities were also increased by canthaxanthin treatment. These enhancing effects on EROD, MROD and 4NP-UGT were clearly detectable at a dose as low as 10 ppm of canthaxanthin in the diet; the induction of QR was only observed in rats fed > or = 100 ppm. Astaxanthin induced the same pattern of enzymes activities as canthaxanthin, but to a lesser extent: its effects on phase I enzymes and 4NP-UGT were observed in rats fed > or = 100 ppm, and QR was not increased. Western blots of microsomal proteins clearly showed the induction of P4501A1 and 1A2 by canthaxanthin and astaxanthin. By contrast, lutein had no effect on the phase I and II xenobiotic-metabolizing enzymes activities measured. Lycopene only decreased NDMAD activity. 3. The two 4-oxocarotenoids canthaxanthin and astaxanthin are substantial inducers of liver P4501A1 and 1A2 in the rat, and coinduce 4NP-UGT and QR, just like polycyclic aromatic hydrocarbon, beta-naphthoflavone or dioxin (TCDD). However, these latter classical P4501A inducers also induce aldehyde dehydrogenase class 3 (ALDH3); this enzyme is not increased, or only marginally, by canthaxanthin and astaxanthin. These two oxocarotenoids form a new class of inducers of P4501A, are structurally very different from the classical inducers quoted above, which are ligands of the AH receptor.

33. Carcinogenesis. 1995 Dec;16(12):2957-63.

Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during the postinitiation phase.

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The modulating effects of dietary feeding of two xanthophylls, astaxanthin (AX) and canthaxanthin (CX) during the postinitiation phase on colon carcinogenesis initiated with azoxymethane (AOM) were investigated in male F344 rats. Animals were initiated with AOM by weekly s.c. injections of 15 mg/kg body wt for 3 weeks and then they were fed the diets containing AX or CX at concentrations of 100 and 500 p.p.m. for 34 weeks. The others contained the groups of rats treated with AX or CX alone and untreated. At the end of the study (week 37), the incidence and multiplicity of neoplasms (adenoma and adenocarcinoma) in the large intestine of rats initiated with AOM and followed by AX or CX containing diet at a high dose (500 p.p.m.) were significantly smaller than those of rats given AOM alone ($P < 0.001$). In addition, AX or CX feeding significantly inhibited the development of aberrant crypt foci induced by AOM. Dietary exposure to AX or CX also decreased cell proliferation activity as revealed by measuring 5'-bromodeoxyuridine-labeling index as crypt cells, colonic mucosal ornithine decarboxylase activity and blood polyamine levels. These results indicate that AX and CX are possible chemopreventers for carcinogenesis of colon in addition to urinary bladder and oral cavity and such effects may be partly due to suppression of cell proliferation.

34. J Nutr. 1995 Oct;125(10):2483-92.

Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen.

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Astaxanthin, a carotenoid without vitamin A activity, enhances T-dependent antigen (Ag)-specific humoral immune responses. We examined carotenoid actions on T-helper (Th) cell activity in a direct manner with reconstitution experiments; spleen Th cells were replaced with Ag-specific Type 1 and Type 2 (Th1 and Th2) Th cell clones. The Ag for the Th1 and Th2 clones were pigeon cytochrome C and rabbit gamma-globulin, respectively. Astaxanthin and beta-carotene augmented the number of IgM antibody (Ab)-secreting cells when unprimed B cells were incubated with Th clones and stimulated with suboptimal doses of Ag specific for each Th clone. The number of IgG Ab-secreting cells were greater with use of in vivo primed B cells than with unprimed B cells in both Th clones. Astaxanthin but not beta-carotene augmented the number of IgG Ab-secreting cells when primed B cells and Th cell clones were stimulated with suboptimal doses of Ag specific for each Th clone. In the presence of optimal doses of Ag for

each Th clone, neither carotenoid augmented the number of Ab-secreting cells. Astaxanthin and beta-carotene may enhance the actions of both Th1 and Th2 cells for humoral immune responses with suboptimal Ag challenges; certain carotenoids may help maintain Ag-mediated immune responses at optimal levels.

35. *Cancer Res.* 1995 Sep 15;55(18):4059-64.

Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin.

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The chemopreventive effects of two xanthophylls, astaxanthin (AX) and canthaxanthin (CX) on oral carcinogenesis induced by 4-nitroquinoline 1-oxide (4-NQO) was investigated in male F344 rats. Rats were given 20 ppm of 4-NQO in their drinking water for 8 weeks to induce oral neoplasms or preneoplasms. Animals were fed diets containing 100 ppm AX or CX during the initiation or postinitiation phase of 4-NQO-induced oral carcinogenesis. The others contained the groups of rats treated with AX or CX alone and untreated. At the end of the study (week 32), the incidences of preneoplastic lesions and neoplasms in the oral cavity of rats treated with 4-NQO and AX or CX were significantly smaller than those of rats given 4-NQO alone ($P < 0.001$). In particular, no oral neoplasms developed in rats fed AX and CX during the 4-NQO exposure and in those given CX after the 4-NQO administration. Similarly, the incidences of oral preneoplastic lesions (hyperplasia and dysplasia) in rats treated with 4-NQO and AX or CX were significantly smaller than that of the 4-NQO-alone group ($P < 0.05$). In addition to such tumor inhibitory potential, dietary exposure of AX or CX decreased cell proliferation activity in the nonlesional squamous epithelium exposed to 4-NQO as revealed by measuring the silver-stained nucleolar organizer regions protein number/nucleus and 5'-bromodeoxyuridine-labeling index. Also, dietary AX and CX could reduce polyamine levels of oral mucosal tissues exposed to 4-NQO. These results indicate that AX and CX are possible chemopreventers for oral carcinogenesis, and such effects may be partly due to suppression of cell proliferation.

36. *Nutr Cancer.* 1995;23(2):171-83.

Effect of carotenoids on in vitro immunoglobulin production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen.

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The effect of carotenoids on in vitro immunoglobulin (Ig) production by peripheral blood mononuclear cells (PBMNC) was examined by employing blood samples from adult volunteers and full-term newborn babies (umbilical cord blood). Under carotenoid-supplemented culture conditions, cells were stimulated by polyclonal stimulants, neoantigens, and a recall antigen (Ag), and IgM, IgA, and IgG levels in the culture supernatant were measured. Beta-carotene and astaxanthin were used as representatives of carotenoids with and without vitamin A activity, respectively. Astaxanthin enhanced IgM production in response to T-dependent Ag (TD-Ag) and a T-dependent polyclonal stimulant. Astaxanthin also augmented IgG production in response to a recall Ag. IgA production without supplemental carotenoids was negligible for all stimuli. However, in carotenoid-supplemented cultures, IgA production was significantly higher in response to a T-dependent polyclonal stimulant than in unsupplemented cultures. IgM and IgA production was augmented at 10^{-8} mol/l astaxanthin, whereas astaxanthin enhanced IgG production in response to a recall Ag at 10^{-10} - 10^{-9} mol/l. Similar enhancing actions of astaxanthin on IgM production were observed in cord blood mononuclear cells (CBMNC), although CBMNC produced less IgM than adult PBMNC. Beta-carotene did not have a significant effect on human Ig production. The carotenoid actions were not demonstrated under serum-free culture conditions; serum is essential for solubilization of carotenoids. In summary, this study has shown for the first time that astaxanthin, a carotenoid without vitamin A activity, enhances human Ig production in response to T-dependent stimuli.

37. *Int J Vitam Nutr Res.* 1995;65(2):79-86.

Vitamin A status and metabolism of cutaneous polyamines in the hairless mouse after UV irradiation: action of beta-carotene and astaxanthin.

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Solar radiations (UV A and B) can cause epidermis photoaging and skin cancers. These frequently irreversible effects result from the in situ generation of free radicals. However, it has been noted that nutritional factors can modulate photochemical damage, in

particular the common carotenoids present in food, which can be considered as potential prophylactic agents against carcinogenesis. We investigated the effect of UV A and B radiations on the skin of the SKH1 hairless mouse fed a diet either lacking in vitamin A or supplemented with retinol, beta-carotene or astaxanthin. The latter is an oxygenated carotenoid (like canthaxanthin) without provitamin A activity and with strong singlet oxygen quenching ability. After analysing of vitamin status of each group (plasma retinol concentrations and hepatic reserves), we searched for UV-induced modifications of polyamine metabolism by measuring epidermal ornithine decarboxylase (ODC) activity and free polyamines concentration (putrescine, spermidine and spermine). In the basal state without irradiation, differences in ODC activity between groups were nonsignificant; but after UV stimulation, ODC increased markedly in the skin of vitamin A-deficient animals, much more than in other groups. Curiously, the addition of astaxanthin or beta-carotene to the regimen containing retinol reduced the protective effect of retinol alone. Regarding polyamines after irradiation, putrescine was significantly increased in the skin of deficient animals, in parallel with ODC activity. However, astaxanthin had a stronger inhibitory effect on putrescine accumulation than retinol, and decreased spermidine and spermine concentrations: this suggests a specific action on transglutaminases.

38. Carcinogenesis. 1994 Jan;15(1):15-9.

Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin.

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The chemopreventive effects of two xanthophylls, astaxanthin (AX) and canthaxanthin (CX), on urinary bladder carcinogenesis induced by N-butyl-N(4-hydroxybutyl)nitrosamine (OH-BBN) was investigated in male ICR mice. Mice were given 250 p.p.m. OH-BBN in drinking water for 20 weeks and after a 1 week interval with tap water, water containing AX or CX at a concentration of 50 p.p.m. was administered during subsequent 20 weeks. Other groups of mice were treated with AX or CX alone or untreated. At the end of the study (week 41), the incidences of preneoplastic lesions and neoplasms in the bladder of mice treated with OH-BBN and AX or CX were smaller than those of mice given OH-BBN. In particular, AX administration after OH-BBN exposure significantly reduced the incidence of bladder cancer (transitional cell carcinoma) ($P < 0.003$). However, the inhibition of the frequencies of such lesions in mice treated with OH-BBN and CX was not significant. Treatment with AX or CX also decreased the number/nucleus of silver-stained nucleolar organizer region proteins (AgNORs), a new index of cell proliferation, in the transitional epithelium exposed to OH-BBN. Preneoplasms and neoplasms induced by OH-BBN, and the antiproliferative potential, was greater for AX than CX. These results indicate that AX is a possible chemopreventive agent for bladder carcinogenesis and such an effect of AX may be partly due to suppression of cell proliferation.

39. J Nutr Sci Vitaminol (Tokyo). 1993 Dec;39(6):607-15.

Inhibitory effect of beta-carotene and astaxanthin on photosensitized oxidation of phospholipid bilayers.

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Large unilamellar liposomes comprising of egg yolk phosphatidylcholine (PC) was exposed to photoirradiation in the presence of methylene blue (water-soluble photosensitizer) or 12-(1-pyrene)dodecanoic acid (P-12, lipid-soluble photosensitizer) to estimate the inhibitory effect of beta-carotene and astaxanthin on photosensitized oxidation of phospholipid bilayers. Without sensitizers, astaxanthin decreased much slower than beta-carotene and other hydrocarbon carotenoids (lycopene, alpha-carotene). Astaxanthin lasted longer than beta-carotene even in the presence of methylene blue or P-12. Decrease of astaxanthin was also much slower than that of beta-carotene when egg yolk PC was replaced by dimyristoyl PC. However, inhibitory effect of astaxanthin was lower than beta-carotene in the case of P-12 sensitized photooxidation. These results suggest that effectiveness of carotenoids as antioxidants on photosensitized oxidation (Type II) in phospholipid bilayers depends on the site of singlet oxygen to be generated, as well as their stability on photoirradiation.

40. Nutr Cancer. 1993;19(3):269-80.

Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation.

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Previously we have shown that astaxanthin, a carotenoid without provitamin A activity, enhances in vitro antibody (Ab) production to sheep red blood cells in normal B6 mice. In this study, we further attempted to examine the mechanisms of this enhancing action of carotenoids on specific Ab production in vitro in relation to different antigen (Ag) stimuli, cytokine production, and T- and B-cell interactions in both normal and autoimmune strains of mice. When the actions of carotenoids were tested in normal strains of mice, we found that astaxanthin enhanced in vitro Ab production to T cell-dependent Ag, but not to T-independent Ag, and did not augment total immunoglobulin production. Astaxanthin exerted maximum enhancing actions when it was present at the initial period of Ag priming. This action of astaxanthin was abolished when T cells were depleted from spleen cell suspensions and appeared to require direct interactions between T and B cells. The results also indicated that carotenoids may modulate the production of interferon-tau in this assay system. When the actions of carotenoids were tested in autoimmune-prone MRL and NZB mice, the enhancing action of astaxanthin on in vitro Ab production was less significant. Furthermore, carotenoids did not potentiate or augment spontaneous Ab and immunoglobulin production by spleen cells in these strains. Taken together, carotenoids without provitamin A activity may be able to augment in vitro specific Ab production to T cell-dependent Ag partly through affecting the initial stage of Ag presentation without facilitating polyclonal B-cell activation or autoantibody production.

41. Arch Latinoam Nutr. 1992 Dec;42(4):409-13.

[Hypercholesterolemic effect of canthaxanthin and astaxanthin in rats]

[Article in Spanish]

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Three groups of male Wistar rats (130-140 g) were fed 30 days with a synthetic diets containing 0.1% of beta-carotene, canthaxanthin and astaxanthin respectively. Another group was fed with a synthetic diet without carotenoids. The results shows that the beta-carotene does not induce change in plasma cholesterol (49, 7 +/- 3.6 mg/dl), but canthaxanthin and astaxanthin induce a significant increase in cholesterol concentration (92.1 +/- 3.6 and 66.5 +/- 5.1 mg/dl). This increase is noted mainly in the HDL fraction of the lipoproteins. Canthaxanthin has more affinity than astaxanthin for the liver, principal site of lipoproteins catabolism. The hypercholesterolemic effect of these xanthophylls is not related to reported mechanisms of carotenoids in mammalian, because beta-carotene does not induce changes in plasma cholesterol.

42. Arch Biochem Biophys. 1992 Sep;297(2):291-5.

Astaxanthin and canthaxanthin are potent antioxidants in a membrane model.

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When the conjugated keto-carotenoids, either astaxanthin or canthaxanthin, are added to rat liver microsomes undergoing radical-initiated lipid peroxidation under air, they are as effective as alpha-tocopherol in inhibiting this process. This contrasts with the effect of beta-carotene, which is a much less potent antioxidant when added in this system, without the addition of other antioxidants.

43. Nutr Cancer. 1991;16(2):93-105.

Studies of immunomodulating actions of carotenoids. I. Effects of beta-carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in in vitro culture system.

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The immunomodulating effects of carotenoids (beta-carotene and astaxanthin) on mouse lymphocytes were studied in in vitro culture system by use of assay for mitogen responses of spleen cells, thymocyte proliferation, interleukin 2 production, and antibody (Ab) production in vitro in response to sheep red blood cells. Changes of cell surface markers on spleen lymphocytes including Ia antigen (Ag), surface immunoglobulin, B220, and Thy-1 Ag were also examined. At a concentration of 10^{-8} M, carotenoids did not show any significant effect on mitogen responses (phytohemagglutinin P and concanavalin A) on murine spleen cells, irrespective of the concentrations of mitogens used. Interleukin 2 production by murine spleen cells was not significantly altered by carotenoids in the culture media (10^{-7} to 10^{-9} M). $[^3\text{H}]$ thymidine incorporation by B6 thymocytes was somewhat

enhanced in the presence of astaxanthin or beta-carotene when cultured in the concentration of 10^6 /ml. At higher concentrations of cells (5×10^6 /ml), such an effect was not observed. In assays of in vitro Ab production in response to sheep red blood cells, B6 spleen cells produced significantly more Ab-forming cells (plaque-forming cells, immunoglobulins M and G) in the presence of astaxanthin (greater than 10^{-8} M) but not beta-carotene. Expression of Ia Ag seemed to be moderately enhanced on both Thy-1+ and Thy-1- spleen cells in the presence of astaxanthin (greater than 10^{-9} M) but not beta-carotene. The expression of Thy-1 and surface immunoglobulin seemed unchanged with the treatment of these carotenoids. These results indicate that immunomodulating actions of carotenoids are not necessarily related to provitamin A activity, because astaxanthin, which does not have provitamin A activity, showed more significant effects in these bioassays and also indicate that such actions of carotenoid demonstrated in this study may be difficult to explain only by its oxygen-quenching capacity.

44. *Physiol Chem Phys Med NMR*. 1990;22(1):27-38.

Inhibition of oxidative injury of biological membranes by astaxanthin.

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The value of astaxanthin, a carotenoid pigment, in the treatment of oxidative injury is assessed. Astaxanthin protects the mitochondria of vitamin E-deficient rats from damage by Fe^{2+} -catalyzed lipid peroxidation both in vivo and in vitro. The inhibitory effect of astaxanthin on mitochondrial lipid peroxidation is stronger than that of alpha-tocopherol. Thin layer chromatographic analysis shows that the change in phospholipid components of erythrocytes from vitamin E-deficient rats induced by Fe^{2+} and Fe^{3+} -xanthine/xanthine oxidase system was significantly suppressed by astaxanthin. Carrageenan-induced inflammation of the paw is also significantly inhibited by administration of astaxanthin. These data indicate that astaxanthin functions as a potent antioxidant both in vivo and in vitro.

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