

The Nutrition News © from IAACN 2010 #4

Publication or duplication of the Nutrition News© without written consent from Barbara Zeitlin Kravets is prohibited by law.

www.iaacn.org
972-407-9089

We are transmitting this in email and also including a WORD attachment of the document with active hyperlinks of references for those who have the ability to receive and download this format. NOTE: Some hyperlinks may be expired or no longer be active.

The NutritionNews © from IAACN 2010 #4

The NutritionNews © 2000 is a gift to IAACN members in good standing from our most esteemed colleague, Barbara Zeitlin Kravets CCN LNC. The NutritionNews © supplies important research information for clinical nutritionists that credibly supports the use of nutrition and we sincerely appreciate Barbara's continuous efforts in supporting IAACN and its members. Links are listed if available within the NutritionNews © and will guide you to the source for the article on the web for added viewing

Barbara Zeitlin Kravets CCN LDN,
Licensed Dietitian Nutritionist,
Certified Clinical Nutritionist,
Medical Nutrition Therapy,
Phone 1 847 870 9514, Fax 1 847 239 6724,

Also send to my other email licnutrition@msn.com
Web site www.findanutritionist.com/practitioners/licnutrition
Editor of The NutritionNews ©, internationally distributed, cutting edge, peer reviewed, journal abstract, email nutrition newsletter

The NutritionNews © 2000 Barbara Zeitlin Kravets CCN LDN 1 23 2010

TABLE OF CONTENTS

Protection against Oxidative Stress, Inflammation, and Apoptosis of High-Glucose-Exposed Proximal Tubular Epithelial Cells by Astaxanthin.....PAGE 01

<http://pubs.acs.org/doi/abs/10.1021/jf9019745>

<http://www.astaxanthin.org/astax.htm>

Protection against Oxidative Stress, Inflammation, and Apoptosis of High-Glucose-Exposed Proximal Tubular Epithelial Cells by Astaxanthin

You Jung Kim†‡, Young Ae Kim‡ and Takako Yokozawa*‡

† Department of Dental Hygiene, Busan Women's College, Busanjin- Gu, Busan, Korea

‡ Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

J. Agric. Food Chem., Article ASAP

DOI: 10.1021/jf9019745

Publication Date (Web): September 4, 2009

Copyright © 2009 American Chemical Society

*Corresponding author (telephone +81-76-434-7670; fax +81-76-434-5068; e-mail yokozawa@inm.u-toyama.ac.jp).

ESSENCE OF ARTICLE

”On the basis of these findings, it was concluded that in PTECs, astaxanthin has a protective efficacy against several deleterious effects caused by high glucose exposure and proposed that astaxanthin should be explored further as a potential antidiabetic remedy for the treatment of diabetic nephropathy.”

ARTICLE

Abstract

Astaxanthin is a carotenoid with powerful antioxidant properties that exists naturally in various plants, algae, and seafood. The purpose of the present study is to examine the protective action of astaxanthin against high-glucose-induced oxidative stress, inflammation, and apoptosis in proximal tubular epithelial cells (PTECs). To assess the efficacy of astaxanthin, several key markers and activities were measured, including lipid peroxidation, total reactive species (RS), superoxide ($\bullet\text{O}_2$), nitric oxide ($\text{NO}\bullet$), and peroxynitrite (ONOO^-), as well as expressions of inflammatory proteins, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), nuclear factor-kappa B (NF- κ B) nuclear translocation, and levels of Bcl2/Bax protein. Results showed that astaxanthin effectively suppressed lipid peroxidation, total RS, $\bullet\text{O}_2$, $\text{NO}\bullet$, ONOO^- , iNOS and COX-2 protein levels, NF- κ B nuclear translocation, and pro-apoptotic Bax, whereas it increased anti-apoptotic Bcl2 protein levels. On the basis of these findings, it was concluded that in PTECs, astaxanthin has a protective efficacy against several deleterious effects caused by high glucose exposure and proposed that astaxanthin should be explored further as a potential antidiabetic remedy for the treatment of diabetic nephropathy.

What is Astaxanthin?

Astaxanthin is a red pigment occurring naturally in a wide variety of living organisms. Although the word astaxanthin may not be commonly encountered in everyday speech, the pigment itself is found in many human foods, and you are quite likely to be consuming it in your diet already. Most crustaceans, including shrimp, crawfish, crabs and lobster, are tinted red by accumulated astaxanthin. The coloration of fish is often due to astaxanthin; the pink flesh of a healthy wild salmon is a conspicuous example. In commercial fish and crustacean farms, astaxanthin is commonly added to feeds in order to make up for the lack of a natural dietary source of the pigment (Torrissen et al. 1989). Not only does astaxanthin provide for pigmentation in these farmed animals, it also has been found to be essential for their proper growth and survival (Torrissen and Christiansen 1995).

Astaxanthin is one of a group of natural pigments known as carotenoids. In nature, carotenoids are produced principally by plants and their microscopic relatives, the microalgae. Animals cannot synthesize carotenoids de novo, thus ultimately they must obtain these pigments from the plants and algae that support their food chains (Britton et al. 1995). Commercial production of astaxanthin from the microalga *Haematococcus pluvialis* is a growing business worldwide, primarily due to the rapid growth of this microorganism and its high astaxanthin content. Other commercial ventures for natural astaxanthin production utilize fermentation of the pink yeast *Xanthophyllomyces dendrorhous* or extraction of the pigment from by-products of crustacea such as the Antarctic krill (*Euphausia superba*). In addition to

production from natural sources, astaxanthin may be chemically synthesized, and synthetic astaxanthin is the major form currently being used in fish feeds (McCoy 1999).

The astaxanthin molecule is similar to that of the familiar carotenoid beta-carotene (Fig. 1), but the small differences in structure confer large differences in the chemical and biological properties of the two molecules. In particular, astaxanthin exhibits superior antioxidant properties to beta-carotene in a number of in vitro studies (Terao 1989; Miki 1991; Palozza and Krinsky 1992; Lawlor and O'Brien 1995). While the positive effects of astaxanthin on farmed fish and crustaceans have been recognized for years, the potential benefits of this powerful antioxidant to human health are only now being revealed.

Fig. 1. Structure of selected carotenoids

References:

Britton, G., S. Liaaen-Jensen, and H. Pfander. (1995) Carotenoids today and challenges for the future. In: Britton, G., S. Liaaen-Jensen, and H. Pfander [eds], Carotenoids vol. 1A: Isolation and Analysis. Basel: Birkhäuser.

Lawlor, S. M. and O'Brien, N. M. (1995) Astaxanthin: antioxidant effects in chicken embryo fibroblasts. *Nutr. Res.*, 15:1695-1704.

McCoy, M. (1999) Astaxanthin market a hard one to crack. *Chem. & Eng. News*, 77: 15-17.

Miki, W. (1991) Biological functions and activities of animal carotenoids. *Pure Appl. Chem.*, 63(1):141-146.

Palozza, P. and Krinsky, N. I. (1992) Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch. Biochem. Biophys.*, 297:291-295.

Terao, J. (1989) Antioxidant activity of beta-carotene-related carotenoids in solution. *Lipids*, 24: 659-661.

Torrissen, O. J. and Christiansen, R. (1995) Requirements for carotenoids in fish diets. *J. Appl. Ichthyol.*, 11:225-230.

Torrissen, O.J., R.W. Hardy, and K.D. Shearer. (1989) Pigmentation of salmonids--carotenoid deposition and metabolism. *CRC Crit. Rev. Aquat. Sci.*, 1: 209-225.

Astaxanthin in Foods

Does astaxanthin occur normally in foods?

Astaxanthin is the most commonly occurring red carotenoid in marine and aquatic animals, and thus occurs naturally in certain human foodstuffs, most importantly in salmon and rainbow trout, to which it imparts a characteristic pink color when present at sufficient levels (Torrissen and Christiansen 1995). Astaxanthin also occurs in shellfish (e. g., lobsters and shrimps), in fish eggs (e. g., salmon roe), and in some other fish species (e. g., red sea bream) (Mera Pharmaceuticals 1999).

Salmon and trout, like other animals, cannot synthesize astaxanthin themselves and must obtain it from their diet--in the wild, from zooplankton (which presumably feed on the microalgae that are the original

producers of the carotenoid), or, in the case of commercial fish feeds used for pen-raised fish, from intentionally added astaxanthin. It has been shown that in fish and crustaceans, astaxanthin is essential for growth and plays a vitamin-like role, and, in fact, astaxanthin is absorbed and deposited in fish flesh more efficiently than are other similar xanthophylls (oxygenated carotenoids) such as canthaxanthin, lutein, or zeaxanthin (Torrissen and Christiansen 1995).

Astaxanthin is thus commonly used world-wide to supplement fish feeds, and is approved in the United States as a safe additive to salmonid fish feed (at up to 80 mg per kg feed) in order to obtain the desired pink to orange-red color (Code of Federal Regulations 21 CFR 73.35). Apart from imparting an attractive color, astaxanthin has been shown to prevent the oxidation of fats in rainbow trout during frozen storage, thus preventing rancidity (Jensen et al. 1998).

Astaxanthin levels in the flesh of Atlantic salmon range from about 4 to 10 mg per kg, whereas levels in wild Pacific salmon can be much higher, with a recent FDA study reporting an average of about 14 mg per kg in coho salmon and about 40 mg per kg in sockeye salmon (Turujman et al. 1997). Thus, a reasonable serving portion of 4 ounces (one-fourth of a pound, 113.4 g) of farmed Atlantic salmon would contain from 0.5 to 1.1 mg of astaxanthin, whereas the same amount of sockeye salmon would contain 4.5 mg of astaxanthin.

References:

Mera Pharmaceuticals, Inc. (1999) Technical Report TR.2102.001. Aquaxan™ HD algal meal use in aquaculture diets: Enhancing nutritional performance and pigmentation.

Jensen, C., Birk, E., Jokumsen, A., Skibsted, L, H., and Bertelsen, G. (1998) Effect of dietary levels of fat, alpha-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. *Zeitschrift f. Lebensmittel-Untersuchung u. Forschung A*, 207(3):189-196.

Torrissen, O. J. and Christiansen, R. (1995) Requirements for carotenoids in fish diets. *J. Appl. Ichthyol.*, 11:225-230.

Turujman, S. A., Wamer, W. G., Wei, R. R., and Albert, R. H. (1997) Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. *J. AOAC Int.*, 80(3):622-632.

Different Chemical Forms of Astaxanthin

Is all astaxanthin the same?

How is synthetic astaxanthin different from natural astaxanthin?

Are there different forms of natural astaxanthin?

Why do we think natural astaxanthin may act differently from synthetic astaxanthin?

Is all astaxanthin the same?

Astaxanthin has chemical features that result in the existence of several forms of astaxanthin:

Stereoisomers. Astaxanthin has two chiral (pronounced "ky-ral"), or asymmetric, centers. These are the carbons numbered 3 and 3' (pronounced "three prime") on the two rings in the structure. One can think of chiral asymmetry as analogous to "handedness". A left hand and a right hand are mirror images of each other--they are similar but not identical, and are not superimposable. Similarly, a chiral center can exist in either of two configurations; the same atoms are bonded to the chiral center, but the three-dimensional arrangements are different and not superimposable. Chemists identify chiral centers as being either R or S (from *rectus* or *sinister*, Latin for "right" or "left"). The two chiral centers in astaxanthin, carbons 3 and 3', can each exist either in the R or the S form, and thus there are a total of three stereoisomers: 3S,3'S, 3R,3'S, or 3R,3'R. The 3S,3'S and 3R,3'R stereoisomers are mirror images of each other and are termed "enantiomers". Each enantiomer has the opposite optical activity of the other, i.e., a solution of a pure enantiomer will rotate plane-polarized light in a direction opposite to that observed for the other enantiomer. The 3R,3'S form is sometimes termed "meso" and is optically inactive because there is a plane of symmetry through the center of the molecule.

Geometric isomers. Carbon-carbon double bonds can have the atoms attached to them arranged in different ways. This arrangement cannot be changed by the atoms twisting or rotating around the bond (since double bonds are not "flexible" in the way single bonds are) without breaking the double bond. If the two largest groups are attached on the same side (looking down the double bond's length) of the double bond, they are termed Z (from *zusammen*, German for "together"). If the two groups are on opposite sides of the double bond, they are termed E (from *entgegen*, German for "opposed"). Older texts may refer to Z as "cis" and E as "trans", however Z and E are the recommended nomenclature today. A double bond may change its geometry from E to Z or vice-versa, but this process requires energy (such as heat) and the breaking and reformation of the double bond. Astaxanthin has several double bonds in the linear portion of the molecule, each of which can potentially exist in the Z or E form. The thermodynamically most stable form of the molecule is all-E ("all-trans") astaxanthin. This is because in the all-E form, the branching methyl (CH₃) groups on the linear portion of the molecule do not compete for space. In nature, Z isomers have been observed at positions 9, 13, and 15, singly or in combination. Thus, several geometric isomers are possible: all-E, (9Z), (13Z), (15Z), (9Z,13Z), (9Z,15Z), (13Z,15Z), and (9Z,13Z,15Z) (Bernhard 1990).

Free or esterified. Astaxanthin has two hydroxyl (OH) groups, one on each terminal ring. These can be "free" (unreacted) hydroxyls, or can react with an acid (such as a fatty acid) to form an ester. If one hydroxyl reacts with a fatty acid, the result is termed a mono-ester. If both hydroxyl groups are reacted with fatty acids, the result is termed a di-ester. Adding a fatty acid to form an ester makes the esterified end of the molecule more hydrophobic. In order of hydrophobicity (difficulty in dissolving in water), we find that di-esters > mono-esters > free.

In summary then, astaxanthin occurs in several different forms which can be classified according to stereoisomers, geometric isomers, and free or esterified forms. All of these forms are found in various natural sources. For example, the predominant stereoisomer of astaxanthin found in krill (*Euphausia superba*, a shrimp-like marine animal) is 3R,3'R (Bernhard 1990), and the majority of this is esterified (Foss et al., 1987). In wild salmon, the predominant stereoisomer is 3S,3'S; in salmon flesh the astaxanthin occurs as the free xanthophyll (Bernhard 1990). The basidiomycete yeast *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) (Gobulev 1995) accumulates astaxanthin as its major carotenoid; in this yeast, astaxanthin occurs as the 3R,3'R stereoisomer and is predominantly esterified. In

the green alga *Haematococcus pluvialis*, astaxanthin occurs as the 3*S*,3'*S* stereoisomer (Bernhard 1990). Astaxanthin from *H. pluvialis* occurs primarily as monoesters (~80%) and diesters (~15%); the predominant fatty acids that make up the esters are C18:1 and C20:0 (Renstrøm and Liaaen-Jensen 1981).

In one study (Østerlie et al. 1999c), rainbow trout (*Oncorhynchus mykiss*) were fed a diet containing synthetic non-esterified astaxanthin in a 1:2:1 mixture of the three stereoisomers 3*S*,3'*S*, 3*R*,3'*S*, and 3*R*,3'*R*; one group of fish received predominantly all-*E* astaxanthin and a second group received an *E/Z* mixture. The all-*E* diet resulted in a greater uptake of astaxanthin, indicating that this geometric isomer is more easily digested by trout. In feces, blood, liver, and fillet, the *R/S* distribution was close to 1:2:1, but in skin and kidney the ratios were about 1:2:2 and 1:2:3, respectively. In rainbow trout, at least, geometric and stereoisomers are distributed selectively in different tissues.

One study on the kinetics of dietary astaxanthin uptake by humans has been reported (Østerlie et al. 1999a, 1999b). In this study, three middle-aged, smoking, male volunteers were given a single olive oil-containing meal with 100 mg of synthetic non-esterified astaxanthin as a defined mixture of all-*E* (all-trans), 9*Z* (9-cis), and 13*Z* (13-cis) geometric isomers (and with the 3*S*,3'*S*:3*R*, 3'*S*:3*S*,3'*S* stereochemical ratio of 1:2:1). The appearance and distribution of astaxanthin was quantified by HPLC analysis of blood samples taken ten times over the 72 hours following the meal. The maximum plasma concentration of astaxanthin was 1.24 mg/L, observed 6 hours postprandially. There was an enrichment of the 13*Z* isomer in plasma; whether this was due to a preferential uptake of the 13*Z* isomers, preferential catabolism of the all-*E* and 9*Z* isomers, *in vivo* isomerization, or some other process was not determined. Distribution of the *E/Z* isomers was consistent among chylomicrons/VLDL, LDL, and HDL lipoprotein fractions. During the absorptive phase, the relative concentration of total astaxanthin in HDL decreased compared to the other lipoprotein fractions. The relative ratio of stereochemical isomers remained unchanged (Østerlie et al. 1999b). The results of this one study indicate that geometric isomerism may be important in the bioavailability of free astaxanthin in humans.

How is synthetic astaxanthin different from natural astaxanthin?

Synthetic astaxanthin is produced as the free (unesterified) xanthophyll and as a 1:2:1 mixture of the three stereoisomers: 3*S*,3'*S*, 3*R*,3'*S*, and 3*R*,3'*R*. The industrial producers of synthetic astaxanthin are Hoffmann-La Roche AG and BASF AG.

Are there different forms of natural astaxanthin?

In its natural state, astaxanthin is usually associated with other molecules (Bernhard, 1990). It is often complexed with proteins, producing an array of colors in different organisms. For example, it is the chromophore in the blue, green, and yellow pigments of lobsters. In other cases, astaxanthin may simply be dissolved in the lipid fraction of complex molecules such as egg lipoproteins, or it may actually be bound chemically to molecules such as fatty acids to form esters. Reddening of some snow algae (Bidigare et al. 1993) and *Haematococcus* is the result of such esters accumulating in cytoplasmic lipid droplets. Less often, because it is not as stable, astaxanthin occurs in cells as a free, unbound molecule.

Whether free or complexed, the atoms comprising an astaxanthin molecule can be oriented in different ways, producing different isomers. The most common geometric configuration in both synthetic and natural astaxanthin is the most thermodynamically stable all-*E* (all-trans) isomer. Astaxanthin from

natural sources tends to occur predominantly as either the 3S,3'S or 3R,3'R form, while the meso (3R,3'S) isomer is the most abundant in synthetic astaxanthin (Bernhard 1990).

Why do we think natural astaxanthin may act differently from synthetic astaxanthin?

All-E isomers are the major geometric isomers in both synthetic and natural astaxanthin (Turujman et al. 1997). However, synthetic astaxanthin is produced as free (unesterified) astaxanthin in a mixture of stereoisomers: the stereoisomers (3R,3'R), (3R,3'S) and (3S,3'S) occur in a ratio of 1:2:1. Natural astaxanthin, on the other hand, is usually esterified and predominantly of (3S,3'S) configuration or, less frequently, mainly (3R,3'R) (Bernhard 1990). In *Haematococcus pluvialis*, astaxanthin occurs as the 3S,3'S stereoisomer and primarily as monoesters (>90%), with diesters comprising ~8% and the free molecule ~1% (Renstrøm et al. 1981). It tends to produce higher pigmentation in rainbow trout compared to synthetic astaxanthin provided at the same dietary concentration (Bowen et al., 1999).

References:

Bernhard, K. Synthetic astaxanthin. The route of a carotenoid from research to commercialization. In: "Carotenoids: Chemistry and Biology," N. I. Krinsky et al. (editors), Plenum Press, New York, 1990, pp. 337-363.

Bidigare, R.R., Ondrusek, M.E., Kennicutt, M.C., II, Iturriaga, R., Harvey, H.R., Hoham, R.W., and Macko, S.A. (1993) Evidence for a photoprotective function for secondary carotenoids of snow algae. *J. Phycol.*, 29: 427-434.

Foss, P., Renstrøm, B., and Liaaen-Jensen, S. (1987) Natural occurrence of enantiomeric and meso astaxanthin. 7. Crustaceans including zooplankton. *Comp. Biochem. and Physiol. B*, 86B: 313-314.

Renstrøm, B. and Liaaen-Jensen, S. (1981) Fatty acid composition of some esterified carotenols. *Comp. Biochem. Physiol. B*, 69:625-627.

Østerlie, M., Bjerkeng, B., and Liaaen-Jensen, S. (1999a) On bioavailability and deposition of bent Z-isomers of astaxanthin. Proceedings of the First International Congress on Pigments in Food Technology, Sevilla, Spain, 24-26 March 1999, pp.157-161.

Østerlie, M., Bjerkeng, B., and Liaaen-Jensen, S. (1999b) Blood appearance and distribution of astaxanthin E/Z isomers among plasma lipoproteins in humans administered a single meal with astaxanthin. Abstract 2A-13. Abstracts of the Twelfth International Carotenoid Symposium, Cairns, Australia, 18-23 July 1999, p. 72.

Østerlie, M., Bjerkeng, B., and Liaaen-Jensen, S. (1999c) Accumulation of astaxanthin all-E, 9Z and 13Z geometrical isomers and 3 and 3' RS optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. *J. Nutr.*, 129:391-398.

Antioxidants and Eye Health

How does oxidation work in diseases of the eye?

Our eyes are our window on the world. Tragically, there are 30 to 50 million cases of blindness worldwide, and a far greater number of people suffering from visual impairment of some kind (Jacques 1999). Two of the leading causes of visual impairment and blindness are age-related macular degeneration and age-related cataracts (Gerster 1991; Jacques 1999). Both diseases appear to be related to light-induced oxidative processes within the eye (Gerster 1989; Snodderly 1995; Christen 1999).

Within the eye, the lens focuses incoming light onto the photosensitive retina, which transmits visual signals to the brain. The lens is made mostly of water and protein; the proteins are normally arranged such that light may pass through unimpeded. Cataracts are areas of the eye's lens that have become cloudy, due to the clumping together of proteins. These cloudy portions may grow over time and reduce the amount of light reaching the retina, resulting in blurred vision, and potentially leading to blindness. Although cataracts may be congenital, or brought on by diabetes, steroid use, or trauma, most are related to aging. Vision loss due to cataract is usually treatable only by surgical removal of the cataract.

The central area of the retina is known as the macula lutea ("yellow spot"). In the middle of the macula lies the fovea, the part of the retina which has the highest density of photoreceptors, hence gives us the highest visual acuity. Although retinal damage can result from injury (such as from staring at the sun), the slow degradation with age of the macular area of the retina, a condition known as age-related macular degeneration or AMD, is a leading cause of blindness (Gerster 1991; Seddon et al. 1994; Snodderly 1995). No treatment is available for most patients (Hampton and Nelsen 1992).

Both cataracts and AMD appear to be linked to the cumulative effects of a lifetime of light-induced oxidation. Both the lens and the retina are exposed continually to light (particularly blue light) and oxygen, which can work together to produce oxygen free radicals. In cataract formation, free radicals appear to impair the lens crystalline proteins, causing them to clump, and also damage proteolytic enzymes that would normally remove the damaged proteins (Gerster 1989). In the retina, with its high levels of oxygen and polyunsaturated fatty acids, peroxidation of membranes likely leads to the death of photoreceptor cells (Gerster 1991). It is therefore not surprising that factors known to be related to oxidation (cigarette smoking, cardiovascular disease, exposure to sunlight, low ocular melanin content) have been shown in epidemiological studies to be related to an elevated risk for AMD (Snodderly 1995; Snow and Seddon 1999).

The human body uses natural defenses against oxidation of these eye tissues, including the antioxidant enzymes glutathione peroxidase, superoxide dismutase, and catalase, as well as the antioxidant vitamins E and C, and the pigment melanin (Gerster 1991). Of particular significance may be the carotenoid pigments lutein and zeaxanthin, which are concentrated in the macula and give it its yellow color (Bone et al. 1985). These pigments are known to absorb blue light and have the potential to quench singlet oxygen (Landrum et al. 1999). Epidemiological studies have shown that a high dietary intake of carotenoids, specifically lutein and zeaxanthin (from spinach, kale, and other leafy green vegetables), is associated with a reduced risk for both nuclear cataracts and AMD (Seddon et al. 1994; Lyle et al. 1999). It has also been shown experimentally that regular consumption of lutein supplements can increase the macular pigment density in the eye, which may potentially reduce the risk for later development of AMD (Landrum et al. 1997). However, little is known about the potential unwanted interactions of carotenoids in the eye when taken in high-dose supplemental form, thus supplementation should be approached with some caution (Snodderly 1995).

References:

- Bone, R.A., J.T. Landrum, and S.L. Tarsis. (1985) Preliminary identification of the human macular pigment. *Vision Res.*, 25:1531-1535.
- Christen, W.G. (1999) Antioxidant vitamins and age-related eye disease. *Proc. Assoc. Am. Physicians*, 111:16-21.
- Gerster, H. (1989) Antioxidant vitamins in cataract prevention. *Z. Ernährungswiss.*, 28:56-75.
- Gerster, H. (1991) Antioxidant protection of the ageing macula. *Age Ageing*, 20:60-69.
- Hampton, G.R., and P.T. Nelsen [eds]. (1992) *Age-related Macular Degeneration: Principles and Practice*. New York: Raven Press. 300 pp.
- Jacques, P.F. (1999) The potential preventive effects of vitamins for cataract and age-related macular degeneration. *Int. J. Vitam. Nutr. Res.*, 69:198-205.
- Landrum, J.T., R.A. Bone, H. Joa, M.D. Kilburn, L.L. Moore, and K.E. Sprague. (1997) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp. Eye Res.*, 65:57-62.
- Landrum, J.T., R.A. Bone, L.L. Moore, and C.M. Gomez. (1999) Analysis of zeaxanthin distribution within individual human retinas. *Meth. Enzymol.*, 299:457-467.
- Lyle, B.J., J.A. Mares-Perlman, B.E. Klein, R. Klein, and J.L. Greger. (1999) Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. *Am. J. Epidemiol.*, 149:801-809.
- Seddon, J.M., U.A. Ajani, R.D. Sperduto, R. Hiller, N. Blair, T.C. Burton, M.D. Farber, E.S. Gragoudas, J. Haller, D.T. Miller, L.A. Yannuzzi, and W. Willett. (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *J. Am. Med. Assoc.*, 272:1413-1420.
- Snodderly, D.M. (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.*, 62(suppl):1448S-1461S.
- Snow, K.K., and J.M. Seddon. (1999) Do age-related macular degeneration and cardiovascular disease share common antecedents? *Ophthalmic Epidemiol.* 6:125-143.

Antioxidants and Neurodegenerative Diseases

How does oxidation work in neurodegenerative disease?

The nervous system, including the brain, spinal cord, and peripheral nerves, is rich in both unsaturated fats (which are prone to oxidation) and iron (Halliwell 1992). The high lipid content of nervous tissue, coupled with its high metabolic (aerobic) activity, makes it particularly susceptible to oxidant damage (Dawson and Dawson 1996). The high level of brain iron may be essential, particularly during development, but its presence also means that injury to brain cells may release iron ions that can lead to oxidative stress via the iron-catalyzed formation of reactive oxygen species (Gerlach et al. 1994).

There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases, including Parkinson's disease (Ebadi et al. 1996), Alzheimer's disease (Markesbery and Carney 1999; Behl 1999), and amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease") (Olanow and Arendash 1994; Simonian and Coyle 1996; Hall et al. 1998) as well as in cases of stroke, trauma, and seizures (Coyle and Puttfarcken 1993; Facchinetti et al. 1998). Decreased levels of antioxidant enzyme activity are found in Parkinson's disease patients (Fahn and Cohen 1992). Evidence of oxidative stress in the form of increased lipid peroxidation and oxidation of DNA bases is seen in the substantia nigra, the area of the brain affected in Parkinson's disease (Jenner 1996). Similar increased lipid peroxidation and oxidation of DNA and proteins are seen in Alzheimer's disease (Retz et al. 1998) and in Huntington's disease (Borlongan et al. 1996). Increases in markers of oxidative stress (e.g., oxidation of proteins or of DNA) are seen in both familial ALS (FALS) and sporadic ALS (SALS) patients (Ferrante et al. 1997). It has been suggested that Alzheimer's disease may be linked to diet, with reduced risk associated with diets high in antioxidants (Grant 1997).

A number of in vitro studies have shown that antioxidants, both endogenous and dietary, can protect nervous tissue from damage by oxidative stress. Uric acid, an endogenous antioxidant, was found to prevent neuron damage in rats, both in vitro and in vivo, from the metabolic stresses of ischemia (oxidative stress as well as exposure to the excitatory amino acid glutamate and the toxic compound cyanide) (Yu et al. 1998). Vitamin E was found to prevent cell death (apoptosis) in rat neurons subjected to hypoxia followed by oxygen reperfusion (Tagami et al. 1998). The same study showed that vitamin E prevented neuronal damage from reactive nitrogen species (Tagami et al. 1998). Both vitamin E and beta-carotene were found to protect rat neurons against oxidative stress from exposure to ethanol (Mitchell et al. 1999). In an experimental model of diabetes-caused neurovascular dysfunction, beta-carotene was found to protect cells most effectively, followed by vitamin E and vitamin C (Cotter et al. 1995).

Most in vivo and clinical studies of the effects of lipid-soluble antioxidant supplementation on neurological diseases have focused on vitamin E. A report in 1991 demonstrated that the rate at which Parkinson's disease progressed to the point when the patient required treatment with levodopa was slowed by 2.5 years in patients given large doses of vitamin C and synthetic vitamin E (Fahn 1991). Although one study reported that high doses of vitamin E resulted in elevated plasma levels but failed to increase vitamin E levels in cerebrospinal fluid (CSF) (Pappert et al. 1996), a later report demonstrated that high doses of vitamin E did result in elevation of CSF vitamin E levels, and possibly brain vitamin E levels (Vatassery et al. 1998). Recently it was shown that the protein responsible for the uptake of Vitamin E is in fact present in brain cells of patients suffering from Vitamin E deficiency or diseases associated with oxidative stress (Copp et al. 1999). In a Dutch study, it was found that the risk for Parkinson's disease was lower for subjects who had higher dietary intakes of antioxidants, particularly vitamin E (de Rijk et al. 1997). The same group reported that a low dietary intake of beta-carotene was associated with impaired cognitive function in a group of persons aged 55-95; no such association was observed for either vitamins C or E (Jama et al. 1996). In an Austrian study, serum concentration of Vitamin E was found to be significantly associated with cognitive function in adults aged 50 - 75 years measured by a standardized test; serum concentrations of lutein/zeaxanthin, cryptoxanthin, canthaxanthin, lycopene, alpha-carotene, beta-carotene, retinol, gamma-tocopherol, and ascorbate had no significant effects (Schmidt et al. 1998). In another study, it was found that patients suffering from Parkinson's disease had consumed less of the small-molecule antioxidants beta-carotene and vitamin C than did non-sufferers of the disease, implying that dietary antioxidants do play a protective role in this disease (Hellenbrand et al. 1996). About 20% of

FALS cases are associated with a mutation in the gene for copper/zinc superoxide dismutase, an important antioxidant enzyme, and in vitro experiments demonstrated that expression of the mutant enzyme in neuronal cells caused cell death, which could be prevented by antioxidant small molecules such as glutathione and vitamin E (Ghadge et al. 1997).

References:

Behl, C. (1999) Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog. Neurobiol.*, 57(3):301-323.

Borlongan, C. V., Kanning, K., Poulos, S. G., Freeman, T. B., Cahill, D. W., and Sanberg, P. R. (1996) Free radical damage and oxidative stress in Huntington's disease. *J. Fla. Med. Assoc.*, 83(5):335-341.

Copp, R. P., Wisniewski, T., Hentatin, F., Larnaout, A., Ben Hamida, M., and Kayden, H. J. (1999) Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res.*, 822(1-2):80-87.

Cotter, M. A., Love, A., Watt, M. J., Cameron, N. E., and Dines, K. C. (1995) Effects of natural free radical scavengers on peripheral nerve and neurovascular function in diabetic rats. *Diabetologia*, 38(11):1285-1294.

Coyle, J. T. and Puttfarcken, P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, 262:689-695.

Dawson, V. L. and Dawson, T. M. (1996) Nitric oxide neurotoxicity. *J. Chem. Neuroanat.*, 10(3-4):179-190.

de Rijk, M. C., Breteler, M. M., den Breeijen, J. H., Launer, L. J., Grobbee, D. E., van der Meché, F. G., and Hofman, A. (1997) Dietary antioxidants and Parkinson disease: the Rotterdam study. *Arch. Neurol.*, 54(6):762-765.

Ebadi, M., Srinivasan, S. K., and Baxi, M. D. (1996) Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog. Neurobiol.*, 48(1):1-19.

Facchinetti, F., Dawson, V. L., Dawson, T. M. (1998) Free radicals as mediators of neuronal injury. *Cell Mol. Neurobiol.*, 18(6):667-682

Fahn, S. (1991) An open trial of high-dosage antioxidants in early Parkinson's disease. *Am J. Clin. Nutr.*, 53(1 Suppl):380S-382S.

Fahn, S. and Cohen, G. (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann. Neurol.*, 32(6):804-812.

Ferrante, R. J., Browne, S. E., Shinobu, L. A., Bowling, A. C., Baik, M. J., MacGarvey, U., Kowall, N. W., Brown, R. H., Jr., and Beal, M. F. (1997) Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J. Neurochem.*, 69(5):2064-2074.

Gerlach, M., Ben-Shachar, D., Riederer, P., and Youdim, M. B. (1994) Altered brain metabolism of iron as a cause of neurodegenerative diseases? *J. Neurochem.*, 63(3):793-807.

- Ghadge, G. D., Lee, J. P., Bindokas, V. P., Jordan, J., Ma, L., Miller, R. J., and Roos, R. P. (1997) Mutant superoxide dismutase-1-linked familial amyotrophic lateral sclerosis: molecular mechanisms of neuronal death and protection. *J. Neurosci.*, 17(22):8756-8766.
- Grant, W. B. (1997) Dietary links to Alzheimer's disease. *Alzheimer's Disease Rev.*, 2:42-55.
- Hall, E. D., Andrus, P. K., Oostveen, J. A., Fleck, T. J., and Gurney M. E. (1998) Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. *J. Neurosci. Res.*, 53(1):66-77.
- Halliwell, B. (1992) Reactive oxygen species and the central nervous system. *J. Neurochem.*, 59(5):1609-1623.
- Hellenbrand, W., Boeing, H., Robra, B. P., Seidler, A., Vieregge, P., Nischan, P., Joerg, J., Oertel, W. H., Schneider, E., and Ulm, G. (1996) Diet and Parkinson's disease. II: A possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case-control study. *Neurology*, 47(3):644-650.
- Jama, J. W., Launer, L. J., Witteman, J. C., Den Breeijen, J. H., Breteler, M. M., Grobbee, D. E., and Hofman, A. (1996) Dietary antioxidants and cognitive function in a population-based sample of older persons: the Rotterdam study. *Am. J., Epidemiol.*, 144(3):275-280.
- Jenner, P. (1996) Oxidative stress in Parkinson's disease and other neurodegenerative disorders. *Pathol. Biol. (Paris)*, 44(1):57-64.
- Markesbery, W. R. and Carney, J. M. (1999) Oxidative stress in Alzheimer's disease. *Brain Pathol.*, 9:133-146.
- Mitchell, J. J., Paiva, M., and Heaton, M. B. (1999) Vitamin E and beta-carotene protect against ethanol combined with ischemia in an embryonic rat hippocampal culture model of fetal alcohol syndrome. *Neurosci. Lett.*, 263(2-3):189-192.
- Olanow, C. W. and Arendash, G. W. (1994) Metals and free radicals in neurodegeneration. *Curr. Opin. Neurol.*, 7(6):548-558.
- Pappert, E. J., Tangney, C. C., Goetz, C. G., Ling, Z. D., Lipton, J. W., Stebbins, G. T., and Carvey, P. M. (1996) Alpha-tocopherol in the ventricular cerebrospinal fluid of Parkinson's disease patients: dose-response study and correlations with plasma levels. *Neurology*, 47(4):1037-1042.
- Retz, W., Gsell, W., Münch, G. Rösler, M., and Riederer, P. (1998) Free radicals in Alzheimer's disease. *J. Neural. Transm. Suppl.*, 54:221-236.
- Schmidt, R., Hayn, M., Roob, B., Reinhart, G., Schmidt, H., Schumacher, M., Watzinger, N., and Launer, L. J. (1998) Plasma antioxidants and cognitive performance in middle-aged and older adults: Results of the Austrian Stroke Prevention Study. *J. Am. Geriat. Soc.*, 46:1407-1410.
- Simonian, N. A. and Coyle, J. T. (1996) Oxidative stress in neurodegenerative diseases. *Annu. Rev. Pharmacol. Toxicol.*, 36:83-116.

Tagami, M., Yamagata, K., Ikeda, K., Nara, Y., Fujino, H., Kubota, A., Numano, F., and Yamori, Y. (1998) Vitamin E prevents apoptosis in cortical neurons during hypoxia and oxygen reperfusion. *Lab. Invest.*, 78(11): 1415-1429.

Vatassery, G. T., Fahn, S., and Kuskowski, M. A. (1998) Alpha tocopherol in CSF of subjects taking high-dose vitamin E in the DATATOP study. Parkinson Study Group. *Neurology*, 50(6):1900-1902.

Yu, Z. F., Bruce-Keller, A. J., Goodman, Y., and Mattson, M. P. (1998) Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *J. Neurosci. Res.*, 53(5):613-625

Antioxidants and Cardiovascular Diseases

How does oxidation work in cardiovascular disease?

Atherosclerosis is a condition where the walls of the arteries are damaged and narrowed by deposits of plaque (cholesterol and other fatty substances, calcium, fibrin, and cellular wastes), eventually blocking off the flow of blood. Plaque deposits can result in bleeding (hemorrhage) or formation of a blood clot (thrombus). When hemorrhage or thrombus blocks the flow of blood through the entire artery, a heart attack or a stroke occurs. High blood levels of cholesterol - particularly the cholesterol carried by low-density lipoprotein ("LDL", a protein found in blood) - are associated with an increased risk of atherosclerosis.

Normal LDL in plasma is not oxidized. Oxidation of LDL is believed to contribute to the development of atherosclerosis (Frei 1995). Macrophage cells preferentially take up oxidized LDL, become loaded with lipids, and convert into "foam cells" (Aviram 1996). Foam cells accumulate in fatty streaks, early signs of atherosclerosis. Humans produce auto-antibodies against oxidized LDL, and the levels of such auto-antibodies are higher in patients with atherosclerosis (Frei 1995).

The identification of LDL oxidation as a key event in atherosclerosis suggests that it may be possible to reduce the risk of atherosclerosis by antioxidant supplementation (Ylä-Herttuala 1991). Vitamin E is the major naturally-occurring antioxidant in human lipoproteins (Bowry et al. 1992). Most circulating carotenoids are associated with lipoproteins in plasma (Clevidence and Bieri 1993). The largest fraction of total carotenoids is found in LDL, as evidenced by the typically yellow color of this lipoprotein fraction (Clevidence and Bieri 1993). The largest fraction of hydrocarbon carotenoids (e.g., beta-carotene and lycopene), as well as most vitamin E and other tocopherols, is transported by LDL (Clevidence and Bieri 1993; Goulinet and Chapman 1997; Oshima et al. 1997), suggesting that these compounds in particular may play an important role in preventing oxidative modification of this lipoprotein fraction. The more polar xanthophylls (oxygenated carotenoids such as lutein, zeaxanthin, canthaxanthin, beta-cryptoxanthin, and capsanthin) are distributed more evenly between HDL and LDL (Clevidence and Bieri 1993; Goulinet and Chapman 1997; Oshima et al. 1997). For example, a Japanese study found that ~70% of hydrocarbon carotenoids (lycopene, alpha-carotene, and beta-carotene) were found in LDL, whereas the polar xanthophylls (capsanthin, lutein, and zeaxanthin) were distributed about equally between HDL and LDL (Oshima et al. 1997). The authors speculated that these polar xanthophylls might be localized at the polar surface of lipoproteins high in phospholipids (as is HDL) (Oshima et al. 1997). Upon subfractionation of LDL particles, it was found that lycopene, beta-carotene and beta-cryptoxanthin are

found mostly in larger, less-dense LDL particles whereas lutein and zeaxanthin are mostly in the smaller, more dense LDL particles (Lowe et al. 1999). Interestingly, the more dense LDL subfractions, which had lower overall carotenoid and vitamin E concentrations, were also more easily oxidized (Lowe et al. 1999).

Epidemiological and clinical data indicate that dietary antioxidants may protect against cardiovascular disease (Frei 1995). Several epidemiological studies have shown an inverse association between serum levels of beta-carotene and other carotenoids and coronary heart disease (reviewed by Kritchevsky 1999). One study found that serum levels of alpha- and beta-carotene and lycopene were 1.9-, 1.7-, and 2.7-fold higher, respectively, in Israeli men than in Czech men; mortality rates, blood pressure, and coronary heart disease rates in the subjects were highest in Czech and lowest in Israeli men (Bobak et al. 1999). However, clinical studies with carotenoid supplementation have been equivocal, and in fact some major clinical trials with beta-carotene supplementation have shown either no or negative effects on chronic diseases such as cardiovascular disease and cancer (reviewed by Mayne 1996 and Kritchevsky 1999). Carotenoids are regarded as good biomarkers for fruit and vegetable dietary intake, but other plant-derived compounds may well play a significant role in health. Still, studies have shown that supplementation with vitamin E (Reaven and Witztum 1993) and other small compounds (including vitamin C, beta-carotene and other carotenoids, and drugs such as probucol) can decrease the susceptibility of LDL to oxidation (Jialal and Fuller 1995); these compounds have in common their antioxidant activity.

Carotid intima-media thickness ("carotid IMT", essentially the thickness of one of the main arteries in the neck) is a measure of asymptomatic early atherosclerosis; in one atherosclerosis risk study, carotid IMT was found to be inversely correlated to the levels of lutein and zeaxanthin, which are xanthophylls (oxygenated carotenoids) regarded as biomarkers of fruit and vegetable intake (Iribarren et al. 1997). Another study found that lutein and cryptoxanthin were twice as high in a population (Toulouse) that had a much lower incidence of coronary heart disease than another group (Belfast), suggesting that such xanthophylls (hydroxycarotenoids) may be useful as antioxidant supplements (Howard et al. 1996).

Few studies have used carotenoids (other than beta-carotene) as anti-atherogenic dietary supplements. One *in vitro* study showed that cell-mediated oxidation of LDL was inhibited by beta-carotene, but enhanced by lutein or lycopene (Dugas et al. 1998). The same researchers later reported that dietary (*i. e.*, *in vivo*) supplementation of 15 mg per day of beta-carotene over four weeks resulted in a 3- to 6-fold increase in the beta-carotene content of LDL; the *in vitro*-tested increase in oxidation resistance of LDL isolated from the subjects was greater than the increase in oxidation resistance seen in LDL enriched *in vitro* 11- to 12-fold with beta-carotene (Dugas 1999). Again, no effect on LDL resistance to oxidation was seen for lycopene supplied as a dietary supplement (Dugas 1999). These results are in contradiction to studies that reported a significant decrease in serum lipid peroxidation and LDL oxidation after three weeks of lycopene dietary supplementation (Agarwal and Rao 1998), and that *in vitro* supplementation of beta-carotene, canthaxanthin, or zeaxanthin inhibited cell-mediated LDL oxidation (Carpenter et al. 1997). A recent large study of the relationship between dietary antioxidant intake and risk for ischemic stroke (as a consequence of atherosclerosis) followed 43,738 men aged 40 -75 years over 8 years (Ascherio et al. 1999). This study found a significant inverse relation between lutein intake and risk for ischemic stroke but this was not independent of other dietary factors. The authors concluded that vitamin E and vitamin C supplements and specific carotenoids did not substantially reduce risk for stroke in the population studied.

References:

- Agarwal, S. and Rao, A. V. (1998) Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. *Lipids*, 33: 981-984.
- Ascherio, A., Rimm, E. B., Hernán, M. A., Giovannucci, E., Kawachi, I., Stampfer, M. J., and Willett, W. C. (1999) Relation of consumption of vitamin E, vitamin C, and carotenoids to risk for stroke among men in the United States. *Ann. Intern. Med.*, 130:963-970.
- Aviram, M. (1996) Interaction of oxidized low density lipoprotein with macrophages in atherosclerosis, and the antiatherogenicity of antioxidants. *Eur. J. Clin. Chem. Clin. Biochem.*, 34(8):599-608.
- Bobak, M., Hense, H. W., Kark, J., Kuch, B., Vojtisek, P., Sinnreich, R., Gostomzyk, J., Bui, M., von Eckardstein, A., Junker, R., Fobker, M., Schulte, H., Assmann, G., Marmot, M. (1999) An ecological study of determinants of coronary heart disease rates: a comparison of Czech, Bavarian and Israeli men. *Int. J. Epidemiol.*, 28: 437-444.
- Bowry, V. W., Ingold, K. U., and Stocker, R. (1992) Vitamin E in human low-density lipoprotein: when and how this antioxidant becomes a pro-oxidant. *Biochem. J.*, 288(Part 2):341-344.
- Carpenter, K. L. H., Van Der Veen, C., Hird, R., Dennis, I. F., Ding, T., Mitchinson, M. J. (1997) The carotenoids beta-carotene, canthaxanthin and zeaxanthin inhibit macrophage-mediated LDL oxidation. *FEBS Letters*, 401: 262-266.
- Clevidence, B. A. and Bieri, J. G. (1993) Association of carotenoids with human plasma lipoproteins. *Methods Enzymol.*, 214:33-46.
- Dugas, T. R., Morel, D. W., and Harrison, E. H. (1998) Impact of LDL carotenoid and alpha-tocopherol content on LDL oxidation by endothelial cells in culture. *J. Lipid Res.*, 39(5):999-1007.
- Dugas, T. R., Morel, D. W., and Harrison, E. H. (1999) Dietary supplementation with beta-carotene, but not with lycopene, inhibits endothelial cell-mediated oxidation of low-density lipoprotein. *Free Radic. Biol. Med.*, 26: 1238-1244.
- Frei, B. (1995) Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. *Crit. Rev. Food Sci. Nutr.*, 35(1-2):83-98.
- Goulinet, S. and Chapman, M. J. (1997) Plasma LDL and HDL subspecies are heterogeneous in particle content of tocopherols and oxygenated and hydrocarbon carotenoids: relevance to oxidative resistance and atherogenesis. *Arterioscler. Thromb. Vasc. Biol.*, 17:786-796.
- Howard, A. N., Williams, N. R., Palmer, C. R., Cambou, J. P. Evans, A. E. Foote, J. W., Marques-Vidal, P. McCrum, E. E., Ruidavets, J. B., Nigdikar, S. V., Rajput-Williams, J., and Thurnham, D. I. (1996) Do hydroxy-carotenoids prevent coronary heart disease? A comparison between Belfast and Toulouse. *Int. J. Vitam. Nutr. Res.*, 66(2):113-118.
- Iribaren, C., Folsom, A. R., Jacobs, D. R., Jr., Gross, M. D., Belcher, J. D., and Eckfeldt, J. H. (1997) Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-

LDL with carotid atherosclerosis: a case control study. *Arterioscler. Thromb. Vasc. Biol.*, 17(6):1171-1177.

Jialal, I. and Fuller, C. J. (1995) Effect of vitamin E, vitamin C, and beta-carotene on LDL oxidation and atherosclerosis. *Can. J. Cardiol.*, 11(Suppl. G):97G-103G.

Kritchevsky, S. B. (1999) β -Carotene, carotenoids and the prevention of coronary heart disease. *J. Nutr.*, 129: 5-8.

Lowe, G. M., Bilton, R. F., Davies, I. G., Ford, T. C., Billington, D., and Young, A. J. (1999) Carotenoid composition and antioxidant potential in subfractions of human low-density lipoprotein. *Ann. Clin. Biochem.*, 36:323-332.

Mayne, S. T. (1996) Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J.*, 10:690-701.

Oshima, S., Sakamoto, H., Ishiguro, Y., and Terao, J. (1997) Accumulation and clearance of capsanthin in blood plasma after the ingestion of paprika juice in men. *J. Nutr.*, 127:1475-1479.

Reaven, P. D. and Witztum, J. L. (1993) Comparison of supplementation of RRR-alpha-tocopherol and racemic alpha-tocopherol in humans: effects on lipid levels and lipoprotein susceptibility to oxidation. *Arterioscler. Thromb.*, 13(4):601-608.

Ylä-Herttuala, S. (1991) Macrophages and oxidized low density lipoproteins in the pathogenesis of atherosclerosis. *Ann. Med.*, 23(5):561-567.

Astaxanthin and Other Health Properties

What are other potential benefits of astaxanthin on health and wellness?

It is clear that astaxanthin is a potent <ANTIOXIDANT< a>carotenoid and, at least in some farmed animals, it provides demonstrable health benefits. Astaxanthin has further been shown to exhibit potential health benefits in numerous tests using laboratory animals. These results offer some indication that astaxanthin may be useful in the maintenance of human health, to the extent that these animal test results can be extrapolated to human beings.

Several studies have shown the effectiveness of astaxanthin as a cancer preventive in rats and mice. For example, Tanaka et al. (1994b) showed that astaxanthin protected mice from urinary bladder carcinogenesis. The investigators fed a known bladder carcinogen to two groups of mice (36 and 33 mice respectively) for 20 weeks. Then, the carcinogen was removed from the diet for both groups. After another week, the second group of mice was fed astaxanthin in the diet for another 20 weeks while the first group did not get any astaxanthin. Histological examination of the mouse bladders indicated that while the group that did not get astaxanthin showed a 42% incidence of bladder carcinoma the astaxanthin group had only an 18% incidence rate. In a second study (Tanaka et al. 1995b) the investigators showed that astaxanthin prevents oral carcinogenesis in rats. The investigators fed a known carcinogen to a group of rats. A second group of the rats was also fed the carcinogen but supplemented with astaxanthin. In this direct comparison they found that the group fed both the carcinogen and astaxanthin had a significantly lower incidence of different types of cancerous growths in their mouths than those rats fed only the

carcinogen. The authors concluded that astaxanthin offered effective protection against oral cancer. They also concluded that the inhibitory effect of astaxanthin on cancer was even more pronounced than that of beta-carotene, which they had tested in a previous study (Tanaka et al. 1994a). A further study by this group (Tanaka et al. 1995a) explored the effect of astaxanthin on colon cancer in male rats. As in the previous studies, groups of rats were fed a known colon carcinogen with or without astaxanthin supplements in the diet. Again, the investigators found a significant ($P < 0.001$) decrease in the incidence of colon cancer in those animals that were given astaxanthin.

Astaxanthin has been shown to significantly influence immune function in a number of in vitro and in vivo assays using animal models. The majority of this work has been carried out by Harumi Jyonouchi and colleagues at the University of Minnesota. Astaxanthin enhances in vitro antibody production by mouse spleen cells stimulated with sheep red blood cells (Jyonouchi et al. 1991), at least in part by exerting actions on T-cells, especially T-helper cells (Jyonouchi et al. 1993). Astaxanthin can also partially restore decreased humoral immune responses in old mice (Jyonouchi et al. 1994). These immunomodulating properties are not related to provitamin-A activity, because astaxanthin, unlike beta-carotene, does not have such activity (Jyonouchi et al. 1991). Studies on human blood cells in vitro have demonstrated enhancement by astaxanthin of immunoglobulin production in response to T-dependent stimuli (Jyonouchi et al. 1995a). Other supporting data on astaxanthin and immune function, including studies on the mechanisms of action involved, may be found in Jyonouchi et al. (1995b), Jyonouchi et al. (1996), Okai & Higashi-Okai (1996), and Tomita et al. (1993).

There is abundant evidence that certain carotenoids can help protect the retina from oxidative damage (Snodderly 1995). A recent study with rats indicates that astaxanthin is effective at ameliorating retinal injury, and that it is also effective at protecting photoreceptors from degeneration (Tso and Lam 1996). The results of this study suggest that astaxanthin could be useful for prevention and treatment of neuronal damage associated with age-related macular degeneration, and that it may also be effective at treating ischemic reperfusion injury, Alzheimer's disease, Parkinson's disease, spinal cord injuries, and other types of central nervous system injuries (Tso and Lam 1996). In this study, astaxanthin was found to easily cross the blood-brain barrier (unlike beta-carotene), and did not form crystals in the eye (unlike canthaxanthin; Tso and Lam 1996).

References:

Jyonouchi H., Hill R. J., Tomita Y., and Good R. A. (1991) Studies of immunomodulating actions of carotenoids. I. Effects of β -carotene and astaxanthin on murine lymphocyte functions and cell surface marker expression in in vitro culture system. *Nutr. Cancer*, 16(2):93-105.

Jyonouchi H., Sun S., and Gross M. (1995) 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. *Nutr. Cancer*, 23(2):171-183.

Jyonouchi H., Sun S., Mizokami M., and Gross M. D. (1996) Effects of various carotenoids on cloned, effector-stage T-helper cell activity. *Nutr. Cancer*, 26(3):313-324.

Jyonouchi H., Sun S., Tomita Y., and Gross M. D. (1995) Astaxanthin, a carotenoid without vitamin A activity, augments antibody responses in cultures including T-helper cell clones and suboptimal doses of antigen. *J. Nutr.*, 125(10):2483-2492.

Jyonouchi H., Zhang L., and Tomita Y. (1993) Studies of immunomodulating actions of carotenoids. II. Astaxanthin enhances in vitro antibody production to T-dependent antigens without facilitating polyclonal B-cell activation. *Nutr. Cancer*, 19(3):269-280.

Jyonouchi H., Zhang L., Gross M., and Tomita Y. (1994) Immunomodulating actions of carotenoids: enhancement of in vivo and in vitro antibody production to T-dependent antigens. *Nutr. Cancer*, 21(1):47-58.

Okai, Y., and K. Higashi-Okai. (1996) Possible immunomodulating activities of carotenoids in in vitro cell culture experiments. *Int. J. Immunopharmacol.*, 18:753-758.

Snodderly, D.M. (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.*, 62(suppl):1448S-1461S.

Tanaka, T., Morishita, Y., Suzui, M., Kojima, T., Okomura, A., and Mori, H. (1994b) Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. *Carcinogen.*, 15:15-19.

Tanaka, T., Makita, H., Ohnishi, M., Hirose, Y., Wang, A., Sato, K., Hara, A., and Ogawa, H. (1994a) Chemoprevention of 4-nitroquinoline-1-oxide-induced oral carcinogenesis by dietary curcumin and hesperidin: comparison with the protective effect of β -carotene. *Cancer Res.*, 54:4653-4659.

Tanaka, T., Kawamori, T., Ohnishi, M., Makita, H., Mori, H., Satoh, K., and Hara, A. (1995a) Suppression of azoxymethane-induced rat colon carcinogenesis by dietary administration of naturally occurring xanthophylls astaxanthin and canthaxanthin during post initiation phase. *Carcinogen.*, 16:2957-12963.

Tanaka, T., Makita, H., Ohnishi, M., Hideki, M., Sato, K., and Hara, A. (1995b) Chemoprevention of rat oral carcinogenesis by naturally occurring xanthophylls, astaxanthin and canthaxanthin. *Cancer Res.*, 55:4059-4064.

Tomita Y., Jyonouchi H., Engelman R. W., Day N. K., and Good R. A. (1993) Preventive action of carotenoids on the development of lymphadenopathy and proteinuria in MRL-lpr/lpr mice. *Autoimmunity* 16:95-102.

Tso, M. O., and T.-T. Lam. (1996) Method of retarding and ameliorating central nervous system and eye damage, U.S. Patent #5527533. Board of trustees of the University of Illinois, United States of America.

Copyright © 2008 by AstaFactor division of Mera Pharmaceuticals, Inc. All rights reserved. Any unauthorized copying, distribution, or adaptation is strictly prohibited. Top of page