5-1997

The other antioxidants: Bioflavonoids and carotenoids

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Abstract

Free radical damage has been associated with atherosclerosis, agerelated macular degeneration, and age-related cataracts. Free radicals cause damage to human tissues through oxidative stress. Protection against free radicals has been found with the use of antioxidants, such as vitamin C, vitamin E, and beta-carotene. Antioxidants neutralize the free radicals and prevent tissue damage; therefore antioxidants are increasingly becoming routine therapies for such diseases as atherosclerosis. Foods may contain other antioxidants such as bioflavonoids and carotenoids other than betacarotene. Research is showing how bioflavonoids and carotenoids inhibit lipid peroxidation and platelet aggregation and thus may be even more beneficial than current therapies for free radical damage.

Degree Type

Thesis

Degree Name

Master of Science in Vision Science

Committee Chair

Diane Yolton

Subject Categories

Optometry

THE OTHER ANTIOXIDANTS: BIOFLAVONOIDS AND CAROTENOIDS

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A thesis submitted to the faculty of the

College of Optometry

Pacific University

Forest Grove, Oregon

for the degree of

Doctor of Optometry

May, 1997

Advisor:

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ABSTRACT

Free radical damage has been associated with atherosclerosis, age-related macular degeneration, and age-related cataracts. Free radicals cause damage to human tissues through oxidative stress. Protection against free radicals has been found with the use of antioxidants, such as vitamin C, vitamin E, and beta-carotene. Antioxidants neutralize the free radicals and prevent tissue damage; therefore antioxidants are increasingly becoming routine therapies for such diseases as atherosclerosis. Foods may contain other antioxidants such as bioflavonoids and carotenoids other than beta-carotene. Research is showing how bioflavonoids and carotenoids inhibit lipid peroxidation and platelet aggregation and thus may be even more beneficial than current therapies for free radical damage.

ACKNOWLEDGEMENTS

We wish to thank Dr. Diane Yolton for her wisdom, support, and tremendous amount of encouragement. We also would like to thank the library staff for their time in locating literature.

Much interest has been shown recently about free radical production and free radical damage in the body. Free radical damage has been associated with coronary heart disease, age-related macular degeneration, cataracts, and cancer. Certain vitamins such as E and C are thought to act as antioxidants, protecting against free radical damage. It is also thought that foods may contain other substances that are protective against free radicals and are possibly even more powerful than the well known antioxidants. This paper will discuss bioflavonoids and carotenoids found in foods that may protect against free radical damage.

FREE RADICALS

Free radicals are molecules that have an unpaired electron in their outermost orbit, thereby making them highly reactive. Oxygen free radicals are common in the body as they are produced by many human metabolic processes (1). In an oxygen free radical, the oxygen molecule is left with an unpaired electron and wants to react with another substance to become more stable. If two radicals react, they bond sharing an electron and the free radicals are eliminated. If a free radical reacts with a nonradical, it pulls away an electron leaving the nonradical now a free radical. This can cause a chain reaction resulting in damaged molecules (2).

Free radicals are produced within cells during many metabolic processes, as well as being produced by environmental changes and through foreign compounds. Foreign compounds that cause free radicals include carbon tetracholoride, which is used in dry cleaning and degreasing, and Adriamycin, an anti-cancer drug (3). The

following are specialized mechanisms of free radical production which can create free radical damage in the body.

Phagocytic cells, when active, create a "respiratory burst" which is composed of free radicals produced from the oxygen they consume. These free radicals are used to destroy any phagocytized material (3)(Figure A).

Hydrogen peroxide (H_2O_2) in combination with metal ions can be converted to hydroxyl radicals ($OH^{\cdot}$), hydroxyl ions (OH-) and oxygen (O_2) (Reaction 1). Radicals are also formed by the Haber-Weiss reaction which uses the metal ion as a catalyst rather than a substrate (Reaction 2)(1).

$$Fe^{2+} + H_2O_2 \text{ ----> } Fe^{3+} + OH^{\cdot} + OH^{-} \quad \text{(Reaction 1)}$$

$$O_2^{\cdot -} + H_2O_2 \xrightarrow[\text{Catalyst}]{\text{Fe salt}} O_2 + OH^{\cdot} + OH^{-} \quad \text{(Reaction 2)}$$

Since metal ions, such as iron and copper, can convert hydrogen peroxide to free radicals, the body keeps the metal ions bound to proteins as an antioxidant mechanism. This prevents free metal ions from aiding in production of free radicals or catalyzing free radical reactions (4).

Much damage has been shown during ischemic reperfusion, possibly in part due to free radicals. During ischemia, adenosine triphosphate (ATP) is catabolized to hypoxanthine. The enzyme,

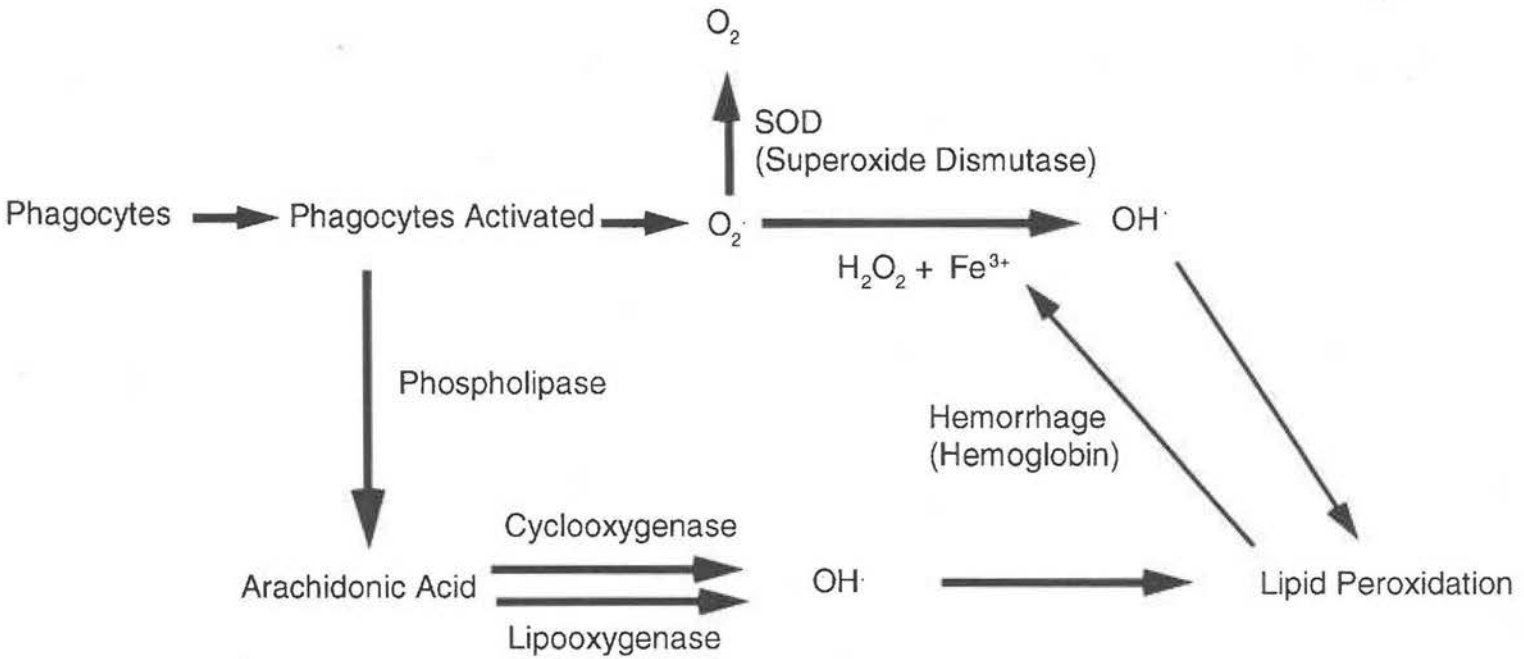

Figure A. Free Radical Production and Damage

xanthine dehydrogenase, is converted to xanthine oxidase by a protease. On reperfusion xanthine oxidase uses hypoxanthine as a substrate, producing free radicals (1).

Ischemia also decreases superoxide dismutase (SOD), which is the enzyme produced in the body that helps catabolize superoxide anion radicals. One study on dogs having coronary artery occlusion showed that by administering superoxide dismutase at the time of reperfusion and continuing for 90 minutes, necrosis of the myocardial cells was decreased by 36% (1). By adding the enzyme that helps break down free radicals, less necrosis of the tissues resulted. This antioxidant mechanism may help in other areas of cell or tissue death.

Another study has shown that animals having the longest lifespan showed higher levels of superoxide dismutase and a lower metabolic rate (1).

FREE RADICAL DAMAGE

Circulatory system

Free radicals produced in the arterial endothelial and muscle cells (1) have been shown to damage vascular endothelium and to affect the regulators for vasculature permeability leading to atherosclerosis (3). Low density lipoproteins (LDLs) are blood lipids that are known to promote atherosclerosis. LDLs are deposited in the endothelial cells of the arterial wall. When free radicals oxidize LDLs, the LDLs in this altered state have different biological properties. The oxidized LDLs can easily be taken up by the macrophages,

thereby leading to foam cell lesions within the arterial wall. These foam cell lesions can grow and eventually cause an atherosclerotic plaque (5,6).

Another mechanism by which oxidized LDLs can contribute to atherosclerosis involves the endothelium derived relaxing factor (EDRF). The endothelial cells produce the relaxing factor (EDRF), which suppresses platelet activity and relaxes smooth muscle cells causing vasodilation (7). Studies have shown that "arteries exposed to oxidized LDL show an unresponsiveness to endothelium-derived vasodilators that is similar to that observed in atherosclerotic vessels" (6). Free radical damage to the endothelium destroys the ability of these cells to secrete the EDRF and this too can initiate atherosclerosis (7). Preventing oxidation, by quenching or neutralizing the free radicals, would protect the endothelial wall of the arterioles and decrease arteriosclerosis.

Bleeding with lysed erythrocytes releases iron molecules that decompose H_2O_2 to free radicals (Figure A). Therefore, it has been shown that bleeding into the eye giving off unbound iron molecules can cause severe retinal degeneration from free radical damage (1). When the iron is bound to another molecule, such as protein, it is unable to participate in free radical production (4).

Smoking and ARMD

The Blue Mountains Eye Study conducted in Australia looked at the correlation between smoking and age-related macular degeneration in 3654 subjects. The results showed a significant association between current smokers and late ARMD as well as early

ARMD. The subjects who had previously smoked during their life had an increased risk of late ARMD but not early ARMD (8).

The Physicians Health Study subjects, 21,157 US male physicians, were assessed for their smoking habits and occurrence of ARMD. The ARMD was found to be dose dependent; therefore, those subjects who smoked the greatest number of packs per day were found to have a greater risk for developing ARMD. A 2-3 times increased risk for ARMD was shown for those who currently smoke or had smoked more than 1 pack per day (9).

A similar study looked at women who smoked and their risk of developing ARMD. 31,843 registered nurses were followed over 12 years. Those subjects that smoked 25 or more cigarettes per day were 2.4 times more likely to develop ARMD. Past smokers of this quantity of cigarettes were twice as likely to acquire ARMD as those who had never smoked (10).

Current theories of how smoking increases the risk of ARMD include the production of free radicals in the retina caused by oxidants found in cigarette smoke and vascular changes in the choroid from lipid peroxidation resulting from free radical damage (9). Any substance found in foods that could neutralize free radicals then could decrease the risk of ARMD.

Age-related Cataracts

Age-related cataracts are formed due to lipid peroxidation from a photochemical reaction which generates superoxide anion radicals. The lens contains many oxygen free radical scavengers to prevent free radical injury, but if not enough free radical

neutralizers are available for the amount of free radicals present, the damage occurs (1).

Cigarette smoking is a risk factor for age-related cataracts. Smoking has been shown to increase the blood levels of products from lipid peroxidation caused by free radicals. A study published in the New England Journal of Medicine measured circulation levels of F2-isoprostanes, compounds produced by the peroxidation of arachidonic acid by free radicals, in smokers and non-smokers. The F2-isoprostane level of smokers was almost twice the level of non-smokers (11).

Another study investigated the incidence of cataracts in smokers using data from the Physicians' Health Study. It controlled other risk factors such as diabetes, alcohol use, blood pressure, physical activity and parental history of heart attacks through a questionnaire. A statistically significant increase in nuclear sclerosis and posterior subcapsular cataracts was found in current smokers who smoke 20 or more cigarettes per day. Past smokers showed an increase in posterior subcapsular cataracts (12).

BIOFLAVONOIDS

Bioflavonoids are a group of substances found naturally in foods giving flavor and color to many fruits, vegetables, wine and teas. The bioflavonoids are made up of phenolic compounds. The phenolic compounds act as antioxidants by donating an electron from their hydroxyl group and conjugated ring to a free radical, which then neutralizes or stabilizes the free radical (13); therefore, the more

hydroxyl groups, the more effective the flavonoid is as an antioxidant.

All bioflavonoids contain the same basic chemical structure, C_6-C_3-C_6. Each flavonoid can be defined by its attached subgroup of sugars, hydroxyl, or methoxyl group(s) at various positions on the basic structure (14).

The flavonoids can be divided into three major subcategories: catechols, anthocyanins, and anthoxanthins. Catechols and tannins, which are the brown pigments, are found in grains, barks, and peelings. Anthocyanins are the dark red, blue and purple pigments we find in fruits, vegetables, and flowers. These include cabbage, blueberries, grapes, and eggplants. Anthoxanthins have almost no pigment or a light yellow pigment. They are found in oranges, tangerines, grapefruits, lemons, carrots, zucchini, and yellow flowers (15).

The plant bilberry, scientifically known as Vacciniium myrtillus, contains bilberry, an anthocyanoside and a powerful antioxidant, comparable to vitamin E (16). Studies have shown that after bilberry intake, night vision improves as the rhodopsin chemical is recycled quicker and more effectively after light exposure (17). Most research on bilberry has been conducted in Europe. Doctors in Europe are prescribing bilberry with vitamin E to improve circulation and protect cell membranes (15).

The anthoxanthins can be broken down into three subgroups:

1. Flavanones - mainly found in citrus fruits and are a very pale yellow to colorless compound.

2. Flavones - the dehydrogenated product of flavanones, thereby making it a more specialized flavonoid. These are found in flowers, seeds, leaves, and roots as pale yellow pigments. In the plants certain flavones act as ultraviolet sensors, and may also exhibit similar functions in humans (18).

Quercetin, a flavone found in various plant rinds as well as in clover and ragweed(15), is one of the strongest antioxidants. Quercetin in tablet form is not recommended and may actually be unsafe, as it is a mutagen. Foods containing quercetin contain the mutagens as well as antimutagens that balance each other (19).

Rutin, a flavone, is composed of two glycosides, hesperidin and eriodectyol. Rutin is water-soluble and is found in grains, specifically buckwheat (15).

3. Flavonols - The flavonols, also pale yellow pigments, have the only group of flavonoids that are a non-phenolic hydroxyl group attached at the C_3 position. Flavonols are precursors of anthocyanins being enzymatically changed through illumination (18).

Anthocyanins and catechols, are found throughout the fruit or vegetable, however, the anthoxanthins are reduced with peeling as it decreases in concentration towards the central core (5,18). The nutrition value of these bioflavonoids is not decreased or lost with cooking and processing except in extreme illumination (18). Limited research is available at this time on any of the synthetic or pill-form bioflavonoids. Foods containing any of the bioflavonoids would be the safest method to receive the beneficial results of the flavonoids at this time.

Szent-Gyorgi conducted studies showing that flavonoids "decreased capillary fragility and permeability", therefore the flavonoids were named vitamin P for permeability in 1936. By 1950 this name was dropped as there was nothing to support that flavonoids were "essential" for normal metabolism or development, which defines a vitamin (5). They are now known as the bioflavonoids not vitamin P, but some literature may still use the vitamin term.

HOW BIOFLAVONOIDS PROTECT AGAINST FREE RADICAL DAMAGE

Bioflavonoids have been shown to quench free radicals and thus can protect against oxidative modifications that are associated with atherosclerosis and coronary heart disease. They can also inhibit platelet aggregation which is associated with atherosclerosis. The following studies describe the association of bioflavonoids and coronary heart disease and atherosclerosis.

Flavonoids appear to be involved in the "French paradox": decreased coronary atherosclerosis with a high fat dietary intake. Since high fat intake is associated with coronary heart disease, researchers wanted to know how the French people with a diet high in fats could have a low level of coronary atherosclerosis. Along with the high fat diet, the French people also consume a high level of red wine. The phenolic substances found in the red wine were tested for their effects on lipid peroxidation and were able to inhibit 60-98% lipid peroxidation depending on the concentration (20). The results of the study suggest that the phenolic flavonoid substances found in the red wine inhibited lipid peroxidation and thus the French have

less atherosclerosis and coronary heart disease than their high fat diet would suggest. This shows how powerful the antioxidant effect of the bioflavonoids can be.

The Seven Countries Study looked at 12,763 men aged 40-59 years old and their lifestyles including flavonoid intake. After 25 years, the individuals with the lowest flavonoid intake showed the highest coronary heart disease mortality and vise versa. The highest correlation with increased coronary heart disease mortality was a high intake of saturated fats, followed by smoking and low flavonoid intake (21).

A similar study assessed 34,789 male health professionals over 6 years. No significant correlation between flavonoid intake and coronary heart disease was found, although in men who had previous cardiovascular disease total flavonoid intake reduced their risk of death from the disease (22).

The Zutphen Elderly Study, conducted in the eastern Netherlands, measured flavonoid intake of 939 men aged 65-84 and assessed the relationship of the flavonoid level to coronary heart disease and myocardial infarction incidence. They first determined the amount of flavonoids present in various fruits, vegetables, and beverages (teas and wines) by sampling from three different supermarkets in their area. Complete information was gathered on the subject's diet and risk factors. Over a five year span the flavonoid intake was significantly related to a decrease in mortality from coronary heart disease. An inverse relationship between flavonoid intake and the incidence of myocardial infarction was borderline (23). This study did have a smaller sample size but also

was confined to a smaller radius, possibly giving better control of the amount of flavonoids actually consumed by the subjects.

A Canadian study also investigated the presumed benefits of red wine in coronary heart disease. The subjects were healthy individuals, free of any non-steroidal anti-inflammatory agents or aspirin that may have affected the platelet activity for the previous two weeks. By measuring the platelet concentrations before and after the flavonoids found in red wine were added, it was found that quercetin inhibited platelet aggregation. Thus, red wine was found to be more beneficial than other alcoholic products because the bioflavonoids can inhibit platelet aggregation (24).

Another study observed flavonol effects on rabbits and cats. The in vivo effects proved to be more powerful than the flavonol effects tested in vitro. Flavonols showed antiplatelet aggregation and antithrombic action through two different mechanisms: free radical scavenging effects and ability to bind to platelet membranes. The flavonols, quercetin and rutin, actually disaggregated a platelet thrombus. The disaggregation doubled in effectiveness when also being treated with a cyclo-oxygenase inhibitor such as aspirin (7). The free radical scavenging effects of the flavonols prevented damage to the endothelial secreting mechanism that produces EDRF.

Like the current daily low dose aspirin therapy, the bioflavonoids have been shown to inhibit platelet aggregation and atherosclerosis. Since the phenolic compounds demonstrate inhibition of both lipid peroxidation and platelet aggregation, they may be more powerful than current therapies.

In addition to epidemiological studies, the flavonoids have been tested in the laboratory for their effects of lipid oxidations. Quercetin was tested for inhibition of lipid peroxidation based on concentration. One micromole of quercetin gave around 25% inhibition. Ninety-five percent inhibition of lipid peroxidation was achieved with five to ten micromoles (25), thereby showing the favorable antioxidant activity of quercetin. When flavonoids were tested for protection of lipid peroxidation, quercetin, baicalein, and myricetin showed to have the strongest in antioxidant effects (26).

To test for free radical reduction and inhibition of lipid peroxidation, flavonoids were added to an in vitro system where gamma-linolenic acid (GLA) stimulated lipid peroxidation in lymphoma cells. Quercetin, the most effective compound, at a concentration of 10-50 micromoles, lowered lipid peroxidation almost 50%, but when 100 micromoles were added to the system, there was the same reduction(50%) of lipid peroxidation. Thus, a very low concentration of quercetin shows the maximum protection. Rutin showed a 37% inhibition of GLA-induced lipid peroxidation. The same study also measured the effects of fat-soluble vitamins, retinol (vitamin A), retinoic acid, and alpha-tocopherol (vitamin E), on GLA-induced lipid peroxidation. The scavenging effect on free radicals was shown to be dose-dependent for the fat soluable vitamins with an overall reduction of lipid peroxidation significantly less than the flavonoids (27). Again bioflavonoid therapies may be more effective than therapy with vitamin E.

Quercetin has also shown to block the synthesis of hepoxilins, normally produced through arachidonate metabolism. When these

hepoxilins are produced they cause endothelial damage of the arterioles leading to arteriosclerosis (26). The flavone, quercetin, inhibits the production of the hepoxilins, therefore inhibiting arteriosclerosis. Other flavonoids may have similar effects but further testing is needed.

When flavonoids were measured for their scavenging effects, the most potent was catechin followed by epicatechin and rutin based on the flavonoid concentration needed to neutralize 50% of the hydroxyl free radicals in the "Fenton system" (28).

When dealing with human subjects it is extremely difficult to keep all control factors equal and only measure one variable, such as flavonoid intake. Although the subjects are closely questioned about their nutrition and lifestyles, it is almost impossible for all subjects to eat the exact diet over a period of years. Based on the in vitro, as well as in vivo data, flavonoids help protect against atherosclerosis. Because the incidence of age-related macular degeneration increases with atherosclerosis, the flavonoid intake should reduce the incidence and severity of this eye disease.

The lens of the eye is continuously producing layers throughout life and therefore is especially susceptible to free radical damage (15). Age-related cataracts have been shown to be caused by free radical damage and lipid peroxidation. In the previous studies discussed, bioflavonoids have scavenged free radicals to prevent free radical damage and inhibited lipid peroxidation. Further studies should be performed on the exact effects of a high intake of bioflavonoids on cataract development.

CAROTENOIDS

Carotenoids are a widely distributed family of tetraterpenes that are synthesized by a large variety of photo synthetic micro-organisms and members of the plant kingdom (29). There are over 563 carotenoids that have been identified, but only 50 have the ability to be enzymatically converted to retinol (vitamin A) in a variety of animals including humans. In nature, carotenoids exist in several forms including hydrocarbons (beta carotene and lycopene) and oxygenated derivatives (oxycarotenoids or xanthophyls). Some carotenoids, such as beta carotene, can and do serve as a large dietary portion of vitamin A in human nutrition (30). Relatively few details of the mechanisms and regulation of metabolism, distribution, tissue make up and tissue deposition of these compounds in humans is known (31). Absorption of both carotenes and xanthophyls is believed to be in a relatively non-specific fashion. The hydrocarbons are thought to be mainly metabolized to retinol in the mucosal cells of the intestinal tract. However, all of the carotenoids are also absorbed during intake. After absorption, the carotenoids are stored in several tissues and organs predominantly the liver and adipose tissue. Noted levels are also found in the adrenals and the testes.

The transportation of carotenoids through the body is done by lipoproteins in the human plasma. Human plasma contains a complex mixture of structurally diverse carotenoids. Carotenoids that are found in substantial concentrations in human plasma are beta and alpha carotene, lutein, lycopene, and beta-cryptoxanthin (32). Research has shown that 75% of blood carotenoids are

hydrocarbons, which bind with low density lipoproteins (LDL) in the human plasma. The remaining 25% of blood carotenoids attach to high density lipoproteins (HDL). Lutein, a xanthophylic carotenoid, is distributed equally between HDL and LDL in the human plasma (33). There are several determinants that influence serum carotenoid levels including dietary intake, destruction in the gastrointestinal tract, efficiency of absorption and metabolism, and rate of tissue uptake. There does not appear to be a definable level of toxicity associated with excessive carotenoid intake except for the deposition of yellow pigment in the skin (34).

Carotenoids are found predominately in fruits and vegetables, with moderate amounts found in grains, fats, diary products, and eggs (35). Low amounts of carotenoids are found in fish and meats (36,37). Tomatoes are a major source of lycopene (hydrocarbon), which is the most abundant carotenoid found in the human serum (38). Spinach along with collard greens are good sources of lutein and zeaxanthin (xanthophylls). When comparing the lutein concentrations in broccoli, brussel sprouts and spinach, there are as much as five times the concentration of lutein as the concentration of beta carotene in those vegetables (table 1)(39). Lutein is the compound that makes corn yellow and gives marigolds their natural yellow color.

HOW CAROTENOIDS PROTECT AGAINST FREE RADICAL DAMAGE

Carotenoids have the ability to interact with various radical species to protect the organism and cells from adverse effects of light, air, and sensitizer pigments (39). The mechanism of how the

GOOD SOURCES OF CAROTENOIDS

Vegetable Fruit	Alpha-Carotene	Beta-Carotene	Lycopene	Lutein
Broccoli	--	700	--	1,900
Brussel Sprouts	--	480	--	1,300
Cabbage	--	80	--	150
Carrots	3600	7,900	--	260
Corn	50	51	--	780
Green Beans	630	44	--	740
Kale	--	4,700	--	21,900
Leaf Lettuce	1	1,200	--	1,800
Peas (Green)	16	350	--	1,700
Spinach	--	4,100	--	10,200
Winter Squash	12	820	--	38
Summer Squash	12	420	--	1,200
Tomatoes	--	520	3,100	100

Carotenoid content micrograms per 100 grams

Mangels, A.R.; Holden, J.M.; Beecher, G.R.; Forman,M.R.; Lanza, E. *J Am Diet Assoc.* 1993;93:284-296.

carotenoids quench the active oxygen species is still unclear. However, some results suggest the free radical's primary site of attack is the terminal 5,6-double bonds in the intact carotenoid molecule (39).

Antioxidant properties exist in carotenoids other than beta carotene. For example, USDA researchers showed that lutein and lycopene are active antioxidants in human sera. In addition lutein has more biological antioxidant activity in the blood serum than any other carotenoid (40). Researchers in Germany have shown that lycopene and lutein are effective in quenching singlet oxygen. As an overall antioxidant, lutein appears to be a key component with the other antioxidants in the prevention of the deleterious effects of the oxidation process from free radicals (41).

In the United States, ARMD is the leading cause of irreversible blindness in adults older than sixty-five years. Oxidative damage of the photoreceptors in the macula is one factor which is thought to cause the development of age-related macular degeneration. Past epidemiological studies have implied that decreased amounts of carotenoid intake leads to an increased incidence of late ARMD (31,42,43). During 1992 through 1994 three important ARMD studies were reported. The first article documented for the first time a connection between reduced incidence of ARMD and high levels of carotenoids in the blood (44). The second paper, separated the different carotenoids that were associated with the risk of ARMD. Lutein, for the first time, was found to be significantly correlated with a reduced risk of macular degeneration (45). The third paper by Seddon et al. defined the important role of carotenoids,

specifically lutein and zeaxanthin in the reduction of ARMD. These researchers observed that adults in the highest quintile of carotenoid intake had a 43% lower risk of ARMD compared with adults in the lowest quintile. When they assessed intake of specific carotenoids, lutein and zeaxanthin were most strongly associated with a decreased risk of ARMD. The foods most associated with decreased risk were spinach and collard greens, both high in lutein and zeaxanthin (43).

In a study by Landrum et al., lutein and zeaxanthin were found to exist in the macula. In a study published in 1997, Landrum et al. furthered their research by plotting out specific concentration differences between lutein and zeaxanthin within the macula. They found on average 70% of the total carotenoids found in controls, was zeaxanthin and this percent was consistent across the retina. Seventeen of twenty-two ARMD eyes had total amounts of lutein and zeaxanthin in central three millimeters of the retina which were below the mean for the control group. For the outer regions of the macula, eleven and twenty-one millimeters from the macula, 15 of ARMD group (68%) were found to be lower in total carotenoid content than the mean corresponding regions in the control group. The distributions of lutein and zeaxanthin were similar in both groups. Macular pigmentation increase appears to be a slow process as demonstrated by the rate of 13.9X10 absorbance units/week. This amounted to 15% increase in pigment level after 72 days of lutein supplementation. The results of this study revealed that slow, significant increases in the level of pigmentation were seen in those subjects with lutein supplementation over a long period of time (46).

Since the carotenoids lutein and zeaxanthin comprise the macular pigment, they are our best defense against excited triplet states and singlet oxygen in the retina. They are believed to serve actively to protect the nerve tissue of the macula, acting as a shield to those tissues posterior to the outer plexiform layer from excessive blue light (47). Photoreceptors, Bruch's membrane, and the RPE are those structures that are most severely damaged in ARMD and are found posterior to the macular carotenoids (46). From the Sedden et al. study, it appears the daily intake of six milligrams of lutein can significantly lower an individuals risk of contracting ARMD (43). The decrease in macular pigmentation has been correlated with the onset of ARMD (46).

Workers at the Hawaii Cancer Center showed that lutein is the most effective carotenoid to inhibit lipid peroxidation which is one oxidation process that occurs in the human serum and the eye (48). A study from the Department of Ophthalmology and Visual Sciences investigated relationships between levels of toxopherols (vitamin E) and carotenoids in the serum and ARMD. The results showed no significant difference between levels of carotenoids and vitamin E and ARMD when controlling levels of cholesterol in the sample. They did however discover that persons with decreased levels of lycopene in their serum had twice as likely a chance of having ARMD (42).

Research has found that oxidative modification of lens proteins mediated by light leads to insolubilization, and thus cataracts. There is some evidence showing the importance of lutein and zeaxanthin in reducing cataract formation. A recent report based on lenses from a seventy-two year old woman has revealed the presence of

carotenoids in the human lens (49). In a study of fifty thousand women, researchers examined cataract formation over an eight year period. The consumption of spinach, which is an ideal source of lutein and zeaxanthin, lead to much lower levels of cataract formation than the consumption of any other vegetables, including carrots, sweet potatoes, and winter squash, which contain high amounts of beta carotene and little lutein (50).

The role of lutein in reduction of cataract formation is farther illustrated in a paper by Yeum et al. Researchers at Tuft University USDA Research Center in Boston. These researchers showed for the first time that lutein and zeaxanthin were found in the lens. They confirmed earlier work that no other carotenoids are found in the eye (51). The researchers at Tuft also reported the presence of lutein and zeaxanthin in the food groups of those nutrients that were earlier believed to reduce and/or prevent cataract formation. The results of a study by Jacques et al. supported the hypothesis that the lens' antioxidant defense may play a role in cataractogenesis particularly when the individual has high plasma levels of two or more of the following vitamins; vitamin C, vitamin E, and carotenoids. These vitamins appear to influence antioxidant status and reduce the risk of cataract formation (52).

SUMMARY

Increasing environmental oxidative stresses are causing the need for more antioxidant protection for our bodies. The powerful antixoidative properties of bioflavonoids and carotenoids may play an important role in prevention of two major eye disorders, age-

related macular degeneration and cataracts, as well as other circulatory diseases. Current antioxidant therapies may be even more beneficial with the use of bioflavonoids and carotenoids.

REFERENCES

1. Southorn, Peter A.; Powis, Garth D. Free radicals in medicine I. Chemical nature and biologic reactions. *Mayo Clin Proc.* 63:381-389;1988.

2. Taber's Cyclopedic Medical Dictionary; FA Davis Company, Philadelphia, PA;1993.

3. Southorn, Peter A.; Powis, Garth D. Free radicals in medicine II. Involvement in Human Disease. *Mayo Clin Proc.* 63:390-408;1988.

4. Halliwell, Barry. Free radicals and antioxidants: A personal view. *Nutr Rev.* 52:253-265;1994.

5. Hertog, MGL.; Hollman, PCH. Potential health effects of the dietary flavonol quercetin. *Eur J Clin Nutr.* 50:63-71;1996.

6. Young, Stephen G.; Parthasarathy, Sampath. Why are low-density lipoproteins atherogenic? *West J Med.* 160:153-164;1994.

7. Gryglewski, Ryszard J.; Korbut, Ryszard; Robak, Jadwiga; Swies, Jozef. On the mechanism of antithrombotic action of flavonoids. *Biochem Pharmacol.* 36:317-322;1987.

8. Smith, Wayne; Mitchell, Paul; Leeder, Stephen. Smoking and age-related maculopathy: The Blue Mountains eye study. *Arch Ophthalmol.* 114:1518-1523;1996.

9. Christen, William G.; Glynn, Robert J.; Manson, JoAnn E.; Ajani, Umed A.; Buring, Julie E. A prospective study of cigarette smoking and risk of age-related macular degeneration in men. *JAMA.* 276:1147-1151;1996.

10. Seddon, Johanna M.; Willett, Walter C.; Speizer, Frank E.; Hankinson, Susan E. A prospective study of cigarette smoking and age-related macular degeneration in women. *JAMA.* 276:1141-1146;1996.

11. Morrow, Jason D.; Frei, Balz; Longmire, Atkinson W.; Gaziano, Michael; Lynch, Sean M.; Shyr, Yu; Strauss, William E.; Oates, John A.; Roberts II, L. Jackson. Increase in circulation products of lipid peroxidation (F_2-isoprostanes) in smokers. *N Engl J Med.* 332:1198-1203;1995.

12. Christen, William G.; Manson, JoAnn E.; Seddon, Johanna M.; Glynn, Robert J.; Buring, Julie E.; Rosner, Bernard; Hennekens, Charles H. A prospective study of cigarette smoking and risk of cataract in men. *JAMA.* 268:989-993;1992.

13. Decker, Eric H. The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as nonessential dietary antioxidants. *Nutr Rev.* 53:49-58;1995.

14. Anonymous. Dietary flavonoids and risk of coronary heart disease. *Nutr Rev.* 52:59-68;1994.

15. Borek, Carmia. Maximize Your Health-Span with Antioxidants. Keats Publishing, Inc.; New Canaan, CT;1995.

16. Beutler, John A. Vaccinium myrtillus. Food ingredient safety review.; March 1994.

17. Sala, D.; Rolando, M., Rossi, P.L.; Pissarello, L. Effect of anthocyanosides on the performance of dark adaptation. *Minerva Oftal.* 21:263-265;1979.

18. Kuhnau, Joachim. The flavonoids. A class of semi-essential food components: Their role in human nutrition. *Wld Rev Nutr Diet.* 24:117-191;1976.

19. Shils, Maurice E.; Young, Vernon R. Modern Nutrition in Health and Disease. Lea and Febiger, Inc.; Philadelphia, PA;1988.

20. Frankel, E.N.; Kanner, J.; German, J.B.; Parks, E.; Kinsella J.E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet.* 341:454-457;1993.

21. Hertog, Michael G.L.; Kromhout, Daan; Aravanis, Christ; Blackburn, Henry; Buzina, Ratko; Fidanza, Flaminio; Giampaoli, Simona; Jansen, Annemarie; Menotti, Alessandro Nedel; Kovic, Srecko; Pekkarinen, Maija; Simic, Bozidar S.; Toshima, Hironori; Feskens, Edith J.M.; Hollman, Peter C.H.; Katan, Martijn B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med.* 155:381-386;1995.

22. Rimm, Eric B.; Katan, Martijn B.; Ascherio, Alberto; Stampfer, Meir J.; Willett, Walter C. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann Intern Med.* 125:384-389;1996.

23. Hertog, Michael G.L.; Feskens, Edith J.M.; Hollman, Peter C.H.; Katan, Martijn B.;. Kromhout, Daan. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet.* 342:1007-1011;1993.

24. Pace-Asciak, Cecil R.; Hahn, Susan; Diamandis, Eleftherios P.; Soleas, George; Goldberg, David M. The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis:

Implications for protection against coronary heart disease. *Clinica Chimica Acta.* 235:207-219;1995.

25. Laughton, Miranda J.; Evans, Patricia J.; Moroney, Michele A.; Hoult, J.R.S.; Halliwell, Barry. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. *Biochem Pharmacol.* 42:1673-1681;1991.

26. Hanasaki, Yukiko; Ogawa, Shunjiro; Fukui, Shozo. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Radic Biol Med.* 16:845-850;1994.

27. Ramananthan, R.; Das, N.P.; Tan, C.H. Effects of gamma linolenic acid, flavonoids, and vitamins on cytotoxicity and lipid peroxidation. *Free Radic Biol Med.* 16:43-48;1994.

28. Pace-Asciak, C.R. Hepoxilins. *Gen Pharmacol.* 24:805-810;1993.

29. Goodman, D.S. Overview of Current Knowledge of Metabolism of Vitamin A and Carotenoids. *JNCL.* 1984;73:1375-1379.

30. Lachance, P. Dietary intake of carotenes and the carotene gap. *Clin. Nutr.* 1988;7:118-122.

31. Parker, R.S. Carotenoids in human blood and tissues. *J Nutr.* 1989;119:101-104.

32. Hankinson, S.; Stampfer, M.J. All that glitters is not beta carotene. *JAMA.* 1994;272:1455-1456.

33. Krinsky, N.I.; Cornwell, D.C.; Oncley, J.L. The transport of vitamin A and carotenoids in human plasma. *Arch Biochem Biophys.* 1958;73:233-246.

34. Alexander, L.J. Ocular vitamin therapy a review and assessment. *Ocular Vitamin Therapy.* Vol. 2, No.4.

35. Heinonen, M. Food groups as the source of retinoids, carotenoids, and vitamin A in Finland. *Int J Vitam Nutr Res.* 1991;61:3-9.

36. Ollilainen, V.; Heinonen, M.; Linkola, E.; Varo, P.; Koivistoinen, P. Carotenoids and retinoids in finnish foods: meat and meat products. *J Food Comp Anal.* 1988;1:178-188.

37. Ollilainen, V.; Heinonen, M.; Linkola, E.; Varo, P.; Koivistoinen, P. Retinoids and carotenoids in finnish foods: fish and fish products. *J Food Comp Anal.* 1989;2:93-103.

38. Mangels, A.R.; Holden, J.M.; Beecher, G.R.; Forman, M.R.; Lanza, E. Carotenoid content of fruits and vegtables: An evaluation of analytic data.

J Am Diet Assoc. 1993;93:284-296.

39. Krinsky, N.I. Antioxidant function of carotenoids. *Free Radic Biol Med.* 1989;7:617-635.

40. Khachik, F.; Beecher, G.R.; Smith, J.C. *J Cell Biochem.* 1995;22:236-246.

41. MiMasco, D.; Kaiser, S.P.; Sies, H. *Arch Biochem Biophys.* 1989;274:532-538.

42. Mares-Perlman, J.A. et al. Serum antioxidants and age-related macular degeneration in a population based case-control study. *Arch Ophthalmol.* 1995;113:1518-1523.

43. Seddon, J.M. et al. Dietary carotenoids, vitamins A, C, and E and advanced age-related macular degeneration. *JAMA.* 1994;272:1413-1420.

44. Eye Disease Control Study Group. Risk factors for neovascular age-related macular degeneration. *Arch Ophthalmol.* 1992;110:1701-1708.

45. Eye Disease Control Study Group. Antioxidant status and neovascular age-related macular degeneration. *Arch Ophthalmol.* 1993;111:104-109.

46. Landrum, J.T.; Bone, R.A.; Kilburn, M.D. The macular pigment a possible role in protection from age-related macular degeneration. *Adv in Pharmacology.* 38:537-555.

47. Schalch W. Carotenoids in the retina : a review of their possible role in preventing or limiting damage caused by light and oxygen. In: Emerit I, Chance B, eds. *Free Radicals and Aging.* Basel, Switzerland: Birkhouser Verlag; 1992:280-298.

48. Zhang, L.H.; Cooney, R.V.; Bertram, J.S. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10 T1/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis.* 12:2109-2114.

49. Daicker, B.; Schiedt, K.; Adnet, J.J. et al. Canthaxanthin retinopathy: A investigation by light and electron microscopy and physiochemical analysis. *Graefe Arch Clin Exp Ophthalmol.* 1987;225:189-197.

50. Hankinson et al. Nutrient intake and cataract extraction in women: a prospective study. *Brit Med J.* 1992;305:335-339.

51. Yeum, K.J. et al. *Invest Ophthalmol Vis Sci.* 1995;36:2756-2761.

52. Jacques, P.F.; Chylack, L.T.; McGandy, R.B.; Hartz, S.C. Antioxidant status in persons with and without senile cataract. *Arch Ophthalmol.* 1988;106:337-340.