

B) SUMMARY OF SCIENTIFIC DATA

1) Changing Dietary Patterns and Lutein Esters Intake

Heber and Bowerman, (2001)¹³ recently reviewed the history of dietary patterns and noted that these were driven by necessity, economics, and more recently, by the selection of foods carefully designed and promoted on the basis of taste, cost and convenience, often without regard to their nutritional and health value. Humans have gradually shifted away from a diverse plant-based diet that provides not only essential vitamins and minerals but also over 25,000 phytochemicals to a diet largely based on refined grains, added oil, sugar predominantly in the form of high fructose corn syrup, and salt.

Results from the most recent USDA Nationwide Food Survey indicate that most Americans eat between two and three servings of fruit and vegetables per day and a minority eat less than one serving a day. (www.5aday.com/html/research/consumptionstats.php#demographics). This is associated with an epidemic of obesity (www.cdc.gov/nccdphp/dnpa/obesity/ and www.cdc.gov/nchs/about/major/nhis/released200202/figure06_2.htm), various cancers and chronic diseases. (www.5aday.org/pdfs/research/health_benefits.pdf). As part of the Consumer Health Initiative for Better Nutrition, on July 29, 2003 FDA, in collaboration with the National Cancer Institute (NCI), issued the following dietary guidance message for consumers: "Diets rich in fruits and vegetables may reduce the risk of some types of cancer and other chronic diseases."¹⁴ Two chronic diseases that should be included are the eye disease disorders of age-related macular degeneration and cataract formation. While fruit and vegetable consumption continue to be suboptimal, and fall far short of the five or more servings recommended by the U.S. Dietary Guidelines, the Food Guide Pyramid, and various government reports, the opportunity exists for foods supplemented with lutein esters to make a positive contribution to the eye health of the American population by reducing some of the risks associated with these eye diseases.

Krinsky et al., (2003) recently reviewed dietary sources of lutein and zeaxanthin. The two foods that have the highest amounts of lutein are spinach and kale. Other major dietary

sources include broccoli, peas, brussel sprouts, and egg yolk. Holden et al., (1999)¹⁵ recently updated the USDA's carotenoid content database. Sommerburg et al., (1998)¹⁶ analyzed 33 fruits and vegetables, two fruit juices and egg yolk and separately quantified lutein and zeaxanthin. Their work demonstrated that fruits and vegetables of various colors can also be consumed to increase dietary intake of lutein and zeaxanthin.

Hadden et al., (1999)¹⁷ analyzed a commercially prepared marigold flower (*Tagetes erecta*) extract for carotenoid composition. Their extract contained 93 % utilizable pigments with <0.3% of lutein oxidation products. The extracted xanthophyll esters consisted of esters of all-trans and cis-isomers of zeaxanthin (4.3%), and the all-trans and cis-isomers of lutein, and lutein esters (94.6%). This matches the composition reported by Cognis.

It is important to note that lutein and zeaxanthin, unlike the provitamin A carotenoids (α - and β -carotene and β -cryptoxanthin) cannot be converted to vitamin A. Their presence in tissues is entirely due to ingestion and they can not be synthesized in any tissue by mammals or other higher animals. Therefore, they do not contribute to increased vitamin A exposure and pose no concerns about altered retinoid signaling as might be the case with β -carotene.

Using USDA's Continuing Survey of Food Intakes by Individuals, Rachman et al., (2002)¹⁸ calculated the total dietary intake of lutein and lutein esters in the USA from natural sources and those proposed for supplementation in foods. The mean intake of lutein is 2 mg/day from natural sources and would be 9.7 mg/day from proposed supplemented foods. Goldbohm et al., (1998)¹⁹ calculated the contribution of various foods to intake of carotenoids. The mean intake of lutein plus zeaxanthin was reported at 2.5 mg/day. Similar data are noted in dietary reference intake tables from the Institute of Medicine (2001).²⁰

Mares-Perlman et al., (2001)²¹ reported in their analysis of the third National Health and Nutrition Examination Survey that American adults, on average, consume 1-2 mg lutein/day. The level of intake varied considerably with intake being particularly high among African-Americans, who averaged 3 mg/day compared with Caucasians and Hispanic-Americans, who consumed one-half that level, on average.

El-Soheemy et al., (2002)²² examined the relation of carotenoids and their concentrations in plasma and adipose tissue as potential biomarkers of dietary intake in a population of women and men. They found that the relative abundance of each carotenoid in the diet was similar to its distribution in plasma but not in adipose tissue. Differences in the relative abundance of each carotenoid in adipose tissue compared with that in plasma and diet, suggest that uptake, storage and turnover rates of each carotenoid differ and depend on factors other than intake.

As will be demonstrated in later sections of this petition, many studies provide evidence for protection against age-related macular degeneration and cataract formation by consumption of the carotenoids lutein and zeaxanthin.

2) Xangold ®Lutein esters conforms to the definition of “substance” in 21 CFR §101.14(a)(2)

Section 101.70(f) requires that the scientific summary of a health claim petition demonstrate that the substance that is the subject of the proposed claim conforms to the definition of the term “substance” in 21 CFR §101.14(a)(2). Lutein esters, which are the subject of this claim, meet the definition of a substance under 21 CFR §101.14(a)(2): “ a specific food or component of food, regardless of whether the food is in conventional food form or a dietary supplement that includes vitamins, minerals, herbs or other similar nutritional substances.” Lutein esters are a form of lutein, a yellow pigment in the chemical family of fat-soluble carotenoids which includes more than 600 naturally occurring members (Moeller et al, 2000). Lutein is found naturally at relatively high levels in green leafy vegetables, and in moderate amounts in yellow-orange fruits and egg yolks. In yellow-orange fruits and in edible flowers lutein is present as a mixture of fatty acid diesters. (www.nal.USDA.gov/fnic/foodcomp/data). Therefore lutein esters are defined components of conventional foods, and meet the FDA definition of a substance under 21 CFR §101.14(a)(2). As mentioned previously, Xangold ® lutein esters are composed of lutein (>93 %) and zeaxanthin (<7 %) diesters.

3) Chemical/Physical/Biological Properties of the Lutein Esters: Lutein and Zeaxanthin

An excellent review of the known science on this subject was recently provided by Krinsky et al., (2003) and is summarized in this section.

A. Chemical Properties

Lutein and zeaxanthin are isomeric dihydroxy-carotenoids in which the ionone ring systems are substituted at the 3 and 3' carbon. They differ in the position of a double bond in one of the ionone rings and each has a variety of stereoisomers. These include the geometrical Z- and E- isomers. The presence of the hydroxyl groups in both lutein and zeaxanthin makes each distinctively more polar than their respective carotene analogs, α - and β -carotene.

In humans, lutein and zeaxanthin are accumulated in lipophilic tissues, such as adipose tissue and are carried in the blood by the lipoproteins, probably in a nonspecific manner similar to cholesterol. They are also distributed equally between LDL and HDL fractions in the blood. The approximate concentration of lutein in human tissue is: serum, 0.1-1.23 μM ; liver, 0.1-3.0 μM ; kidney, 0.037-2.1 μM ; and lung 0.1-2.3 μM (National Academy of Sciences, 2001). In the human retina, the concentration of these pigments reaches its highest levels, between 0.1 and 1mM, thus providing solid evidence for their active uptake and storage (National Academy of Sciences, 2001).

B. Physical Properties

The ability of carotenoids to absorb light arises from the presence of a conjugated polyene chain. Light absorption is a function of the conjugation in the polyene chain. Both lutein and zeaxanthin have nine conjugated double bonds in the polyene chain.

C. Biological Properties of Lutein Esters

1. Light Filtration; Chromatic Aberration

Because of their very high absorptivity, lutein and zeaxanthin in the inner retina form a very efficient filter for blue-light that reaches the back of the eye. The macular pigment is chiefly accumulated in the Henle fiber layer which is composed of the photoreceptor axons that

overlay the photoreceptors themselves (Snodderly, (1995)²³. These macular carotenoids attenuate blue light prior to its reaching the delicate functional structures including the photoreceptors, the retinal pigment epithelium, and the underlying choriocapillaris of the eye. Landrum et al., (1997)²⁴, concluded that it is highly probable that this reduction in blue-light intensity, which can be as great as 90% and is normally about 40%, could significantly reduce the oxidative stress on the retina and may be sufficient to account for the reduction in risk of AMD that has been reported in some epidemiological studies. Schlach et al., (1999), also reviewed studies providing evidence that have now demonstrated that the macular carotenoids protect the retina from damage due to exposure to blue-light. Bernstein et al., (1997)²⁵ demonstrated that retinal tubulin binds macular carotenoids and this is postulated to play an important role in their photoprotective effects against the progression of age-related AMD. A recent review by Sies and Stahl (2003)²⁶ also provides an excellent overview of the protective effects of lutein and zeaxanthin on the eye.

Chromatic aberration arises in optical systems when refraction of different wavelengths occurs to different extents, producing multiple overlapping images most often characterized by the presence of colored fringes and a loss of image sharpness. The reduction of blue fringes is a result of the absorption of blue light by the macular pigment and has been suggested as a probable advantage resulting from the presence of these yellow pigments (Krinsky et al. 2003) although the role of the macular pigment in improving retinal image has also been questioned by McLellan et al., (2002)²⁷.

2. Membrane Properties

While the precise location of the macular pigment molecules that are present in the Henle fiber layer of photoreceptor axons is not known, observations by Snodderly et al., (1984)²⁸ in primates using polarized light reveal that the molecules are highly organized.

An alternative hypothesis is that the carotenoids are protein bound within the photoreceptor axon. A recent report by Yemelyanov et al., (2001)²⁹, provided the first direct evidence for the existence of specific xanthophyll-binding protein(s) in the vertebrate retina and macula. Interestingly, upon binding with the protein, lutein exhibits a shift in its absorption

maximum to 460nm, the same wavelength maximum that is determined psychophysically for the macular pigment. However, this same red shift is also observed when lutein is incorporated into liposomes leaving open the question of where the macular pigments are localized in the cellular structures of the nerve cell.

3. Antioxidation and Pro-oxidation

Carotenoids, including lutein and zeaxanthin, are well known for their natural antioxidant potential (Krinsky et al., (2003)). They are easily oxidized, losing an electron from the polyene chain to form a radical cation. Schlach (1992)³⁰ reviewed the possible role of carotenoids in limiting damage to the retina caused by light and oxygen. He proposes that the accumulation of lutein and zeaxanthin from the diet in the macula is not accidental, but that their presence may prevent or limit damage via their physiochemical properties and their capability to quench oxygen free radicals and singlet oxygen, which are generated in the retina as a consequence of the simultaneous presence of light and oxygen.

Careful analysis of rod outer segments isolated from the perifoveal and peripheral regions of the retina by both Rapp et al., (2000)³¹, and Sommerburg et al., (1999)³² clearly demonstrate that lutein and zeaxanthin are present in these cellular structures. These observations are important because an essential requirement for the antioxidant function of lutein and zeaxanthin is that they are present in the outer segments and retinal pigment epithelium where the effects of oxidation appear to produce the greatest damage. The hypothesis that lutein and zeaxanthin function as antioxidants is consistent with the observation that the retina is a highly aerobic tissue with an exceptionally high rate of metabolism, and further, there is significant evidence that AMD results from oxidative degradation and radical processes occurring in the outer segments and retinal epithelium (RPE) Beatty et al., (2000)³³.

Khachik et al., (1992)³⁴ reported on the isolation and structural elucidation of the geometrical isomers of lutein and zeaxanthin in extracts from human plasma. Bone et al., (1997)³⁵ further mapped the distribution of macular pigment stereoisomers in the human retina and proposed a pathway to account for the presence of the non-dietary carotenoid, meso-zeaxanthin, via conversion from lutein in the macula. In a review by Furr and Clark (1997)³⁶, the intestinal absorption and tissue distribution of carotenoids were reported to be influenced by

dietary factors with the more polar carotenoids (xanthophylls) being more efficiently absorbed. Landrum et al., (1999)³⁷ reported on the distribution of three stereoisomers of zeaxanthin in the retina. Their findings support the hypothesis that lutein and/or zeaxanthin undergoes oxidation in the retina followed by a nonstereospecific reduction to generate the stereoisomers noted and are consistent with an antioxidant function for the macular carotenoids. Bernstein et al., (2001)³⁸, identified the spectrum of carotenoids and metabolites in human donor eyes. They evaluated extracts from ocular tissues [retinal pigments, epithelium/choroids (RPE/choroid), macula, peripheral retina, ciliary body, iris, lens, vitreous, cornea and sclera]. Nearly all structures examined with the exception of vitreous, cornea and sclera had quantifiable levels of dietary (3R, 3'R, 6'R)-lutein, zeaxanthin, their geometrical isomers, as well as their metabolites, (3R, 3'S, 6'R)-lutein (3-epilutein) and 3-hydroxy- $\beta\epsilon$ -caroten-3'-one. In addition, human ciliary body revealed the presence of monohydroxy carotenoids and hydrocarbon carotenoids, while only the latter group was detected in human RPE/choroids. Uveal structures (iris, ciliary body, and RPE/choroids) account for approximately 50% of the eye's total carotenoids and approximately 30% of the lutein and zeaxanthin. In the iris, these pigments are likely to play a role in filtering out phototoxic short-wavelength visible light, while acting as antioxidants in the ciliary body. Further, they postulated that both of these mechanisms, light filtering and antioxidant radical trapping, may be operative in the RPE/choroid. In addition, carotenoids must transit the RPE in order to be transported from the circulation into the retina..

In addition to the principal retinal components of the macular pigment, lutein, zeaxanthin, and meso-zeaxanthin, there are also several other carotenoids present in the retina. The presence of oxidative metabolites in the retina, although not proof of carotenoid antioxidant activity, is at least consistent with such a hypothesis. Similarly, the presence of meso-zeaxanthin, 3S, 3'S-zeaxanthin and epilutein, all of which could be formed via an oxidation/reduction pathway, is consistent with an active participation in oxidative metabolism within the retina (Krinsky et al., 2003).

Sujak et al., (1999)³⁹, showed that both lutein and zeaxanthin had the same efficacy in protecting lipid membranes against free radical attack.

Beatty et al., (2000) provided an excellent review of current research on the role of oxidative stress in the pathogenesis of age-related macular degeneration and highlighted the susceptibility of the retina due to its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light. Khachik et al., (1997)⁴⁰ evaluated carotenoids of a composite of 58 pairs of human retinas and a monkey retina. In addition to lutein and zeaxanthin, several oxidation products thought to be derived from them are present in the retinal extracts suggesting that lutein and zeaxanthin may act as antioxidants to protect the macula. Further, their ability to absorb short- wavelength visible light is also an important role.

Accumulation of lipofuscin by retinal pigment epithelium is considered to be a potentially important feature in the pathogenesis of age-related macular degeneration. Sundelin and Nillson (2001)⁴¹ reported significantly lower amounts of lipofuscin in rabbit and calf RPE cells treated with several different antioxidants including lutein and zeaxanthin. It is suggested that the carotenoids play a protective role through the chain breaking abilities in peroxidative reactions of lipid membranes and quenching of free radicals.

Filtering of blue light by the macular pigments lutein and zeaxanthin has been proposed as a possible mechanism of protection. Junghans et al., (2001)⁴² provided further data to support the efficacy of carotenoids in filtering blue light in the lipophilic membrane. Filter efficacy was in the order lutein > zeaxanthin > β -carotene > lycopene. Shaban and Richter (2002)⁴³ reviewed the data on retinoid metabolite toxicity to the eye and affirmed the positive role of lutein and zeaxanthin in protecting the retina. Nillson et al., (2003)⁴⁴ recently provided additional data on the protective effects of lutein and zeaxanthin in reducing lipofuscin formation and blue light damage in cultured RPE cells.

Krinsky (2002)⁴⁵ provides an excellent overview on possible biological mechanisms and evidence for a protective role of the two xanthophylls lutein and zeaxanthin in the macular region of the retina. One of the most promising new insights and approaches to the study of age-related macular degeneration has been reported by Crabb et al., (2002)⁴⁶ as it relates to oxidative damage of the retina and the formation of drusen. Using proteome analysis they have demonstrated that protein modifications exist in drusen, including crosslinks and other modifications that can be generated from the oxidation of lipids and carbohydrates and result in

drusen formation. These data strongly support the hypothesis that oxidative injury contributes to the pathogenesis of AMD and that oxidative protein modification may have a critical role in drusen formation. An accompanying editorial by Bok (2002)⁴⁷ as well as a report in *The Lancet* (2002)⁴⁸ by Lawrence support these findings.

4. Role of Oxidation in Cataract Formation

Head (2001)⁴⁹ provided a review of natural therapies for ocular disorders. Factors that contribute to cataract development include aging, smoking (Christen et al., (1992))⁵⁰, exposure to UV-B and ionizing radiation (Taylor, 1999)⁵¹, toxic metabolites of oxygen as initiators of lipid peroxidation in the retina (Bhuyan and Bhuyan, 1984)⁵², increased body weight (above 22 percent body fat) and central obesity (Schaumberg et al., 2000)⁵³, and family history.

Spector and Garner (1981)⁵⁴ demonstrated that oxidation of lens protein is an early event in the development of cataract and that high levels of hydrogen peroxide are present in the aqueous fluid of some cataract patients. They postulated that this could permeate the lens to produce oxidation due to low levels of reduced glutathione (GSH). The data of Sweeney and Trescott (1998)⁵⁵ support an impediment to glutathione diffusion in older, normal human lenses, which over time may allow oxidative modifications of protein to take place in the nucleus, resulting ultimately in nuclear cataract. Altomare et al., (1997)⁵⁶ provided additional evidence for an alteration of the protein redox status of the eyes of patients affected by diabetes mellitus with a greater oxidation of lens and vitreous proteins. Garner et al.,(2000)⁵⁷, provides further support for the formation of hydroxyl free radicals in the human lens and their relationship to the severity of nuclear cataract. Saxena et al., (2000)⁵⁸ provided evidence that transition metal-catalyzed oxidation is partially responsible due to copper binding of the protein and the generation of free radicals.

Since oxidation of lens proteins is clearly a part of the pathophysiology of cataracts, it is not surprising that antioxidants may help prevent the formation of cataracts. Yeum et al., (1995, 1999)^{59 60} analysis of human cataractous lenses showed that the newer, epithelial/outer cortex layer had some of the carotenoids (lutein and zeaxanthin), tocopherol, and retinol (approximately 3-, 1.8-, 1.3-fold higher respectively) than the older, inner cortex/nuclear portion. Other studies

by Bates et al.,(1996)⁶¹ have quantified significant levels of lutein, zeaxanthin, and alpha- and gamma- tocopherol in the lens. Dietary modification with moderate lutein supplementation (6mg/day for 5 weeks) elevated serum levels in an older adult population (Nelson et al., (2003)⁶² and lowered serum 8-hydroxy-2'-deoxyguanosine and total alkenals (malondialdehyde and 4-hydroxynonenal), markers for oxidation stress.

Some of the mechanisms involved in the formation of diabetic cataracts differ from age-related cataracts in that the accumulation of polyols within the lens is a primary contributing factor (Head, (2001)). Davies and Morland (2002)⁶³ demonstrated that the ocular media of diabetic persons are abnormal, with increased lens and reduced macular pigment densities which suggest oxidative stress as a causative factor in diabetic maculopathy.

Epidemiological and clinical studies are presented in a later section of this petition to support a positive claim for lutein esters as a source of lutein in decreasing/delaying the incidence of age-related cataracts.

5. Bioavailability of Lutein Esters

The July 17, 2002 GRAS Notification Letter to FDA for Xangold[®] Lutein Esters submitted by Cognis Corp., reviews results from several human clinical studies measuring serum lutein levels following consumption of lutein supplements (acute and chronic) and the bioavailability of lutein from lutein esters or unesterified lutein. The results from all of these studies indicate that both lutein esters and lutein are bioavailable sources of lutein. Stahl and Sies (1996)⁶⁴ reported that, in general, the ingestion of esters of lutein or zeaxanthin does not result in their presence in serum or in chylomicrons, suggesting that cleavage of carotenoid esters occurs prior to release into the lymphatic circulation. Granado et al., (1998)⁶⁵ reported detection of lutein esters in serum after lutein ester supplementation but stated that this indicated a “ceiling effect” on the saturation transport capacity for xanthophylls, and lutein may have been re-esterified *in vivo* because of the unusual dietary conditions.

Lutein bioavailability was recently determined by Bowen et al., (2002)⁶⁶, comparing lutein diester and unesterified lutein formulations as they might be incorporated into dietary

supplements. They concluded that lutein from the lutein ester formulation is equally well absorbed as compared to unesterified lutein. They concluded that dissolution in the formulation is an important factor in lutein bioavailability. Roodenburg et al., (2000)⁶⁷ determined that the amount of fat in the diet can influence lutein uptake and bioavailability from lutein esters. Consumption of higher amounts of fat resulted in a higher plasma lutein response. Finally, assessments in a group of Type 1 diabetics showed no effect of Type 1 diabetes mellitus on the absorption and depletion rate of lutein in serum (Granado et al., (2002))⁶⁸.

In summary, although chronic diseases of the eye are multifactorial in causation, there is a substantial body of scientific evidence establishing the biological plausibility and mechanisms for the prevention or mitigation of the conditions leading to age-related macular degeneration and cataract formation from consumption of the lutein esters as a source of lutein leading to serum increases of lutein and zeaxanthin and increases in macular pigment optical density.

4) Role of Lutein Esters in Improving Macular Pigment Density and Reducing the Incidence of Age-Related Macular Degeneration

A.Observational Studies: AMD

Excellent reviews supporting a relationship between lutein and zeaxanthin and AMD have been prepared by a number of qualified experts. These studies are summarized in Table 2. Snodderly (1995)⁶⁹ reviewed evidence for protection against AMD by carotenoids and antioxidant vitamins, highlighting that many cases of AMD should be preventable with smoking cessation, adequate intake of fruits and vegetables and avoidance of excessive sunlight. Landrum et al., (1997) reviewed studies on the macular pigmentation, how it functions to protect the retina and how long-term lutein supplementation of individuals having low levels of macular pigmentation could result in a significant increase in the level of pigmentation within the macula. Moeller et al., (2000) reviewed studies assessing the potential role of the dietary xanthophylls lutein and zeaxanthin, and their association with a reduced risk of cataract and AMD. Landrum and Bone (2001)⁷⁰ provided a minireview of lutein, zeaxanthin, their role as predominant carotenoids of the macular pigment, and their functional role in protection against light-induced retinal damage and AMD. Hammond et al., (2001)⁷¹, also provided a minireview on lutein and

zeaxanthin in the retina and lens and their possible positive acute and chronic effects on human visual performance. Emphasis is given to evidence that lutein and zeaxanthin could improve human visual performance through acute optical effects both at the retina and the crystalline lens. The relationships between dietary intake, serum and retinal concentrations of lutein and zeaxanthin were studied in a large group of volunteers (Curran-Celentano et al., (2001))⁷². Macular pigment optical density (MPOD) was positively correlated with dietary intake of lutein and zeaxanthin and their presence in serum. They stated that MPOD could also be influenced by other factors as well including biological factors such as individual absorption profiles and day-to-day variations in blood concentrations contributing to the attenuation of diet-serum correlations. In a symposium titled: Can Lutein Protect Against Chronic Disease, Curran-Celentano et al., (2002)⁷³ reviewed in vivo assessments of macular pigment detection techniques and their impact on monitoring pigment status. They concluded that while some evidence indicates that the macular pigment carotenoids lutein and zeaxanthin may protect the retina and lens and improve optical function, more data and assessment are required that delineate the optimal level of these xanthophylls, their mechanisms of action, and whether they improve vision in patients with eye diseases. This statement reflects the fact that a good deal of the data developed on macular pigment density were gathered by studying healthy individuals free of disease and at a low risk of developing eye disease. The authors do emphasize that lutein does appear to have a protective effect on the retina and that there are wide variations in the concentration of macular pigment density. This could be due in part to the low average dietary intake of lutein and zeaxanthin. Also, non-dietary factors such as genetics, demographics and lifestyle characteristics influence macular pigment density. Mares-Perlsman et al., (2002)⁷⁴ reviewed the body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. They concluded that while some evidence is supportive for the role of lutein and zeaxanthin in eye diseases, it is insufficient to support a recommendation that increased levels of lutein and zeaxanthin will confer an important health benefit. These researchers are interested in gaining a better understanding of the biologic mechanisms by which lutein and zeaxanthin mediate their possible role in preventing disease and also the effects of their consumption, independent of other nutrients in fruits and vegetables.

Hammond and Johnson (2002)⁷⁵ provided a comprehensive review of studies relating to dietary prevention and treatments of age-related macular degeneration. They provided

supportive data to the growing evidence indicating that dietary lutein and zeaxanthin may prevent age-related losses in visual acuity leading to AMD. Krinsky et al., (2003), reviewed biological mechanisms of the protective role of lutein and zeaxanthin in the eye.

A review by Pauleikhoff et al., (2001)⁷⁶ also focused on macular pigment and age-related macular degeneration and on cumulative light damage by oxidative processes in the macular photoreceptors as an environmental cofactor for development of AMD.

Most recently, Bartlett and Eperjesi (2003)⁷⁷ reviewed seven randomized controlled trials investigating the role of nutritional supplementation in AMD. Positive effects were seen in the majority of these studies including the Lutein Antioxidant Supplementation Trial (LAST) and are discussed later in this petition. Granado et al., (2003)⁷⁸ reviewed the nutritional and clinical relevance of lutein in human health. Results for supplementation studies with lutein predominantly show improvement in visual function including dark adaptation, visual acuity, foveal sensitivity, contrast sensitivity and glare recovery.

It is evident that the macular pigment density is important to visual function. Hammond et al., (1997,1998)^{79 80}, investigated the relationship between retinal carotenoid (i.e. macular pigment) and lens optical density. A small group of males (aged 24 to 31 years) and females (aged 24 to 31 years) were compared with older subjects, (females aged 55 to 78 years) and males (aged 48 to 82 years). Lens density was observed to increase with age, there was an inverse relationship between macular pigment density and lens density suggesting that lutein and zeaxanthin, or other dietary factors with which they are correlated, may retard age-related increases in lens density and decline in visual function. Significant differences in macular pigment density were found for different colored irises (Hammond et al., 1996)⁸¹, suggesting that this may be the result of either the evolution of a shared tendency to accumulate melanin and carotenoids due to similar environmental pressures (e.g. light and oxygen); and/or macular pigment might be depleted due to the tendency for eyes with light irises to transmit more light than eyes with dark irises, thus causing oxidative stress. Cigarette smoking depresses MPOD in the retina and decreases macular pigment density leading to age-related macular degeneration (Hammond et al., 1996)⁸². Although dietary factors play a role in macular pigment density, a limited study in monozygotic twins found no significant difference between macular pigment

density and carotenoids in the blood or diet (Hammond et al., 1995)⁸³. Sex differences in macular pigment optical density (measured psychophysically) and plasma levels of lutein and zeaxanthin were examined. Males had a 38% higher macular pigment density ($P < 0.001$) than females despite similar plasma carotenoid concentrations (Hammond et al., 1996)⁸⁴.

A number of studies have examined the relationship between lutein and zeaxanthin in the eyes, serum and diet of human subjects, and their relationship to AMD. Bone et al., (2000)⁸⁵ showed positive associations between dietary lutein and zeaxanthin intake and serum concentration and macular pigment density. Mares-Perlman et al., (2001), using analysis of lutein and zeaxanthin in the diet and serum and their relationship to age-related maculopathy (ARM) in the Third National Health and Nutrition Examination, showed a direct relation of dietary levels to one type of early ARM (soft drusen) but these differed by race and age. Higher levels of lutein and zeaxanthin were related to lower odds for pigmentary abnormalities, one sign of early ARM (odds ratios among persons in high vs. low quantities = 0.1, 0.3) and of late ARM (odds ratio = 0.1, 95% confidence interval = 0.0, 0.9) after adjustments for age, gender, alcohol use, hypertension, smoking, and body mass index.

Broekmans et al., (2002)⁸⁶ in a study of 376 volunteers examined macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. Macular pigment density was 13% higher in men than in women ($P < 0.05$) but serum and adipose tissue concentrations of lutein were significantly higher in women. Thus, the association between macular pigment density and increased lutein levels seems stronger for men than women. Rock et al., (2002)⁸⁷ identified correlates of dietary lutein and zeaxanthin intake and determinants of serum lutein and zeaxanthin in a heterogeneous community-based sample of adults aged 18-92 years at three sites ($n = 2286$). Demographic characteristics, body mass index and lifestyle factors were significantly associated with dietary lutein and zeaxanthin intake and while serum concentration varied, every 10% increase in dietary lutein and zeaxanthin was associated with a 2.4% increase in serum lutein concentration.

Beatty et al., (2001)⁸⁸ evaluated macular pigment density and the risk for age-related macular degeneration in subjects from a Northern European population ($n = 46$), in ages 21 to 81 with healthy maculae and in 9 healthy eyes known to be at high-risk of AMD because of

advanced diseases in the fellow eye. An age-related decline in the optical density of macular pigment was noted in subjects with no ocular disease. Healthy eyes predisposed to AMD had significantly less macular pigment than healthy eyes at no risk. These findings are consistent with the hypothesis that supplemental lutein and zeaxanthin may delay, avert, or modify the causes of the disease. Gale et al., (2003)⁸⁹ investigated the relation between plasma concentrations of lutein and zeaxanthin and AMD in a group of elderly men and women (n = 380), aged 60 to 75 years. Risk of AMD was increased in people with the lowest plasma concentrations of lutein plus zeaxanthin (odds ratios 1.9, 95% CI (0.9-3.5) and in those with the lowest concentrations of lutein (OR 1.7, 95% CI 0.9-3.3), but neither were statistically significant. The increased risk of AMD was statistically significant for zeaxanthin (OR 2.0, 95% CI = 1.0-4.1).

A key point in assessing plasma appearance and retinal presence of lutein is uptake. Yao et al., (2000)⁹⁰ quantified the plasma appearance of a physiological dose of lutein in humans using carbon-13 lutein. The appearance and disappearance of [¹³C]-lutein in plasma after ingestion of a dose similar to that absorbed from foods was quantified. This approach using physiological tracer doses demonstrated a direct correlation between uptake and metabolism to what is observed from dietary fruits and vegetables. Specifically, they were able to completely define the plasma appearance and disappearance of lutein after a dose equivalent to that observed from a single vegetable serving.

Gellerman et al., (2002)⁹¹ used in vivo resonance Raman scattering spectroscopy to reliably measure macular carotenoid pigments in young and aging human retinas. Using this technique, Bernstein et al., (1998 and 2002)^{92 93} quantified the macular carotenoids in normal subjects and in age-related macular degeneration patients (n = 93 AMD eyes from 63 patients and n = 220 normal eyes from 138 subjects). Carotenoid Raman signal intensity declined with age in normal eyes (P < 0.001) and average levels of lutein and zeaxanthin were 32% lower in AMD eyes versus normal elderly control eyes. Patients who had begun consuming supplements containing high doses of lutein (>4mg/day regularly for two months) after initial AMD diagnosis had average pigment levels that were in the normal range and were also significantly higher than in AMD patients not consuming the supplements.

In a small prospective study Schweitzer et al., (2000)⁹⁴ evaluated measurable spectrophotometric parameters of the fundus of patients with early and late age-related maculopathy (ARM), their children, and normal controls. They found that xanthophyll pigment is reduced only in late ARM and that autofluorescence and oxygen saturation are different between ARM patients. They were unable to identify a genetically based predisposition to the parameters evaluated. Falcini et al., (2003)⁹⁵ reported in a limited short-term study of patients with early AMD, that receiving oral supplementation of lutein (15mg) for 180 days was associated with significant changes in the macula and suggested that increasing the level of retinal antioxidants may influence macular function early in the disease process, as well as with normal aging. Dagnelie et al., (2000)⁹⁶ conducted a small pilot study for 26 weeks examining the effect of lutein supplementation (40mg/day for 9 weeks, 20mg/day thereafter) on visual acuity. They showed short-term vision improvements in acuity.

Table 2. Observational studies of lutein (L) and zeaxanthin (Z), Macular Pigment Density and AMD

Study reference	Study type	No. of subjects	Outcome
Hammond et al., 1997	Observational	51	Age-dependent, inverse relationship between macular pigment and lens density and L and Z
Hammond et al., 1998	Observational	37	High macular pigment density associated with visual acuity
Hammond et al., 1996	Observational	95	Difference in macular pigment density and eye color
Hammond et al., 1996	Observational	68	Cigarette smoking reduces macular pigment density
Hammond et al., 1995	Observational	88	Males had significantly higher macular pigment density than females and L and Z plasma levels positively correlated in males
Broekmann et al., 2001	Observational	376	Association between macular pigment density, serum L and Z and adipose L concentrations stronger in men than women
Rock et al., 2002	Observational	2786	Demographics, body weight index, dietary L and Z intake, smoking, race/ethnicity all influence serum L and Z concentration
Beatty et al., 2001	Observational	55	Age related decline in macular pigment density and significantly less macular pigment in healthy eyes predisposed to risk
Gale et al., 2003	Observational	380	Significantly greater risk for AMD in people with low plasma L and Z
Yao et al., 2000	Observational	4	Uptake of ¹³ C lutein similar to that from vegetables
Gellermann et al., 2002	Observational	NS	Quantitative method for screening of macular carotenoid pigment in large populations
Bernstein et al., 2002	Observational	63 228	Low levels of L and Z in human macula consistent with AMD risk
Schweitzer et al., 2000	Observational	97	L and Z reduced only in late AMD, no genetically-based predisposition seen
Falsini et al., 2003	Observational	30	Dietary supplementation of lutein improves function of macular pigment
Dagnelie et al., 2000	Observational	16	Visual acuity improved with lutein supplementation in some patients with retinal degeneration

B. Case-Control Studies with Lutein and Zeaxanthin and AMD

A synopsis of these studies is found in Table 3. The Eye Disease Case-Control Study Group (1993)⁹⁷ reported that an increased intake of carotenoids including lutein/zeaxanthin, was associated with the largest decreased risk for AMD. In a subsequent study, Seddon et al. (1994)⁹⁸ reported that intakes of lutein and zeaxanthin from dark green, leafy vegetables were associated with a very significant decrease in the relative risk of developing AMD. It is important to note that 6 mg/day median intake is the level of lutein in the upper quartile of this study where a significant effect was noted. Conversely, Mares-Perlman et al., (1995)⁹⁹ in a nested case-control study within a population-based cohort (Beaver Dam Eye Study) found no relationship between levels of tocopherols and carotenoids in the serum and AMD.

Bone et al., (2001)¹⁰⁰ reported in a case-control study of macular pigment in donor eyes with and without AMD that there is an inverse association between risk of AMD and the amounts of lutein and zeaxanthin in the retina. Flood et al., (2002)¹⁰¹ could not find evidence of protection from dietary antioxidant or zinc uptake and the incidence of early AMD in a population-based cohort study (The Blue Mountain Eye Study). The authors state that their population may have been exposed to insufficient doses to confer protection. Snellen et al., (2002)¹⁰², reported in a case-control study of Dutch patients that the prevalence rate of AMD in patients with low antioxidant intake and low lutein intake was almost twice as high as that in patients with high intake and a clear dose-response relationship was shown.

Table 3. Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measures	AMD/ ARM measures	Effects observed
Beatty et al., 2001, UK	Case control	46 controls including 9 controls matched to cases, 9 cases with advanced AMD in one eye only	21-81	FFQ, dietary L and Z	AMD in one eye as choroidal neovascularization with soft drusen and/or pigmentary changes, MPOD	<p><u>Controls vs. AMD cases:</u> Dietary L and Z (mg/1000 kcal): 0.016 vs. 0.021, p=0.48 Dietary L (mg/1,000 kcal): 0.012 vs. 0.016, p=0.47 Dietary Z (mg/1,000 kcal): 0.004 vs. 0.005, p=0.58</p> <p><u>Controls vs. AMD-at-risk eye, MPOD:</u> 0.311 vs. 0.147, p=0.015</p> <p><u>Summary:</u> sample size is too small to draw conclusions about diet and AMD; MPOD not corrected with diet in healthy volunteers; however, associations of AMD and MPOD were strong</p>

Table 3 (cont.). Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measures	AMD/ ARM measures	Effects observed
Bone et al., 2001, US	Autopsy, eyes from National Disease Research Interchange	56 AMD (21 M, 34 W) 56 controls (24 M, 27 W) 224 eyes total	82 AMD 77 control	L and Z in retina (HPLC)	AMD diagnosis from donor information sheet	<p><u>Risk of AMD based on L and Z per unit area:</u></p> <p>Inner retina (highest vs. lowest quintiles: 9.08 vs. 2.05 pmol/mm²): OR=0.07 (95% CI: 0.02-0.30, p=0.0005)</p> <p>Medial retina (highest vs. lowest quintiles 0.497 vs. 0.097 pmol/mm²): OR=0.07 (95% CI: 0.05-0.64, p=0.019)</p> <p>Outer retina (highest vs. lowest quintiles: 0.135 vs. 0.031 mol/mm²): OR=0.18 (95% CI: 0.05-0.64, p=0.027)</p> <p><u>Summary:</u> provides support for inverse association of AMD risk with L and Z in retina; however, information from donors lacking as to the extent and type of AMD and other well known risk factors such as smoking</p>
Christen et al., US, Physicians Health Study I	Prospective, initial enrollment 1982, duration 1982-95	21,120 men, 279 incident cases of ARM, including 68 exudative	40-84 y at entry	Supplement use at baseline (use of vitamin A was exclusion criteria)	Early signs, Exudative	<p>Any vitamin C: RR=1.03 (95% CI:0.71-1.50, ns)</p> <p>Any vitamin E: RR=0.87 (95% CI: 0.53-1.43, ns)</p> <p>Any multivitamin: RR=0.90 (0.68-1.19, ns)</p> <p><u>Summary:</u> No significant relationship of supplement use with ARM in the total sample, or in subgroup of those with exudative ARM or in smokers</p>

Table 3 (cont.). Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measures	AMD/ ARM measures	Effects observed
Curran-Celentano et al., 2001, US, Indiana	Cross-sectional	278, assume no AMD	18-50	FFQ	Macular pigment optical density (MPOD)	<p>Serum L: $r=0.26$, $p<0.001$ Serum Z: $r=0.20$, $p<0.001$ Serum β-carotene: $r=0.06$, ns Serum total carotenoids: $r=0.13$, $p<0.01$ Diet L and Z: $r=0.20$, $p<0.001$ Dietary fat: $r=0.03$, ns Dietary energy: $r=0.03$, ns</p> <p><u>Summary:</u> MPOD consistently associated with both serum and dietary L and Z</p>
Mares-Perlman et al., 2001, US, NHANES III	Case control 1988-1996	8,222 (860 with early ARM, 47 with late ARM)	≥ 40	Diet (FFQ) and serum L and Z	Early ARM (macular soft drusen, pigmentary abnormalities), late ARM	<p>Median L and Z quintiles (highest vs. lowest: levels corresponding to quintiles not given, mean dietary intake was 1.48 mg/d, 50th percentile for serum was 0.38-0.40 $\mu\text{mol/L}$):</p> <p><u>Macular soft drusen:</u> Serum, OR=1.0 (95% CI: 0.6-1.5, p not given) Diet, OR=1.4 (95% CI: 1.0-1.8, p not given)</p> <p><u>Pigmentary abnormalities:</u> Serum, OR=0.9 (95% CI: 0.5-1.5, p not given) Diet, OR=0.7 (95% CI: 0.4-1.3, p not given)</p> <p><u>Late ARM:</u> Serum, OR=0.6 (95% CI: 0.2-1.9, p not given) Diet, OR=0.5 (95% CI 0.1-1.6, p not given)</p> <p><u>Summary:</u> dietary and/or serum L and Z were not protective against early signs of ARM or late ARM, but low levels were associated with other risk factors for ARM such as smoking, obesity, low dietary βcarotene, high fat intake, low zinc intake, and being female</p>

Table 3 (cont.). Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measurements	AMD/ARM measures	Effects observed
Smith et al., 1999, Australia, Blue Mountains Eye Study	Case control 1992-94	2,900 72 late ARM 240 early ARM	~49	FFQ, selected nutrients, foods, and supplements	Early ARM, Late ARM	Median carotene quintiles (highest vs. lowest: 12,519 vs. 2303 vs. JU/day) Late ARM, OR=0.73 (95% CI: 0.27-1.99, p=0.62) Early ARM, OR=0.66 (95% CI: 0.39-1.10, p=0.10) <u>Summary:</u> no associations of ARM with dietary antioxidants from diet alone or including supplements, or from selected antioxidant rich vegetables <u>Baseline large drusen related to past median intakes of:</u> Pro-vitamin A carotenoid quintile (highest vs. lowest: 1,626 vs 475 µg/1,000kcal): OR=0.53 (95% CI: 0.3-1.0, p=0.03) L and Z quintile (highest vs. lowest: 1,006 vs. 294 µg/1,000kcal): OR=0.93 (95% CI: 0.5-1.8, p=0.86) Vitamin C, diet and supplements, quintile (highest vs. lowest: 139.8 vs. 23.0mg/1,000kcal): OR: 0.83 (95% CI 0.4-1.6, p=0.01) Zinc, diet and supplements, quintile (highest vs. lowest: 7.42 vs. 3.87 mg/1,000 kcal): OR=0.72 (95% CI: 0.4-1.4, p=0.28) <u>Summary:</u> several antioxidants in past diet inversely associated with baseline large drusen, but not with baseline pigmentary abnormalities or 5-yr incidence of early ARM; few relationships of any sign of ARM with baseline diet
VandenLanzenberg et al., 1998, US, Beaver Dam Eye Study ¹⁰³	Retrospective, 1978-80 and prospective, 1988-90 and 1993-1995 (5 yr incidence)	1,586 (114 early ARM at baseline, 103 new early ARM at follow-up)	43-84	FFQ for past and baseline diet, selected antioxidants from food and supplements, 13 measures of antioxidant and zinc intake	Baseline early ARM (larger drusen, pigmentary abnormalities). Prospectively any early ARM lesion	

Table 3 (cont.). Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measures	AMD/ ARM measures	Effects observed
Age-Related Eye Disease Study Research Group, 2001, US	Intervention, enrolled 1992-98, followed through 2001, average follow-up 6.3 yrs	3640, 2 outcomes were progression to an AMD event or visual acuity loss	69, 55-80	4 treatments: placebo, antioxidants, zinc, or antioxidants and zinc; antioxidants/d: 500mg vitamin C, 400 IU dl- α -tocopherol, and 15mg β -carotene; zinc was 80mg zinc as zinc oxide and 2mg copper as cupric oxide	3 categories of increasing amount and/or severity of lesions; too few outcomes in the least severe category, so analyses based on the 2 more severe categories	<p><u>Progression to advanced AMD (from Categories 3 and 4):</u> Antioxidants vs. placebo: OR=0.76 (99% CI: 0.54-1.05, p=0.03) Zinc vs. placebo: OR=0.70 (99% CI: 0.50-0.97, p=0.005) Antioxidants and zinc vs. placebo: OR=0.66 (99% CI: 0.47-0.93, p=0.001)</p> <p><u>Risk of loss of visual acuity (from Categories 3 and 4):</u> Antioxidants vs. placebo: OR=0.86 (99% CI: 0.65-1.17, p=0.23) Zinc vs. placebo: OR=0.82 (99% CI: 0.61-1.09, p=0.07) Antioxidants and zinc vs. placebo: OR=0.75 (99% CI: 0.55-1.02, p=0.017)</p> <p><u>Summary:</u> provides support for role of these antioxidants and/or zinc in several measures of visual function; numerous secondary outcomes also were assessed with most in the direction of a benefit of one or more of the treatments</p>
Seddon et al., 1994	Case control	356	59-80	FFQ	Exudative AMD	Those with highest uptake of carotenoids had 43% lower risk of AMD OR=0.57 (95% CI: 0.35-0.92, p<0.02)
Mares-Perlman et al., 1995	Nested case control	170	43-84		Grading Funder Photographs	Levels of carotenoids not correlated with AMD

Table 3 (cont.). Case-Control Studies of relationship of lutein (L) and zeaxanthin (Z) intake, blood and/or tissue levels and AMD

Study reference	Study design	Sample size, no. of cases	Average age (yrs)	Dietary, blood, and/or tissue measures	AMD/ ARM measures	Effects observed
Flood et al., 2002, Blue Mountain Eye Study	Cohort	2,335	54	FFQ	Retinal Photographs	No evidence of protection associated with usual dietary antioxidant intake
Snellen et al., 2002	Case control	72 cases 66 controls	60+	FFQ	AMD	Prevalence of AMD in patients with low L intake was twice as high as in patients with high intake OR=1.7 (95% CI: 0.8-3.7) and OR=2.4 (95% CI: 1.1-5.1)

C. Interventional Studies of Lutein, Zeaxanthin, Lutein Esters and AMD

There have been several studies demonstrating that dietary lutein, zeaxanthin, or lutein esters as a source of lutein in food or dietary supplement form can increase the amount of macular pigment. These studies are summarized in Table 4.

Hammond et al., (1997)¹⁰⁴ demonstrated via dietary intervention with 60g spinach (10.8mg lutein, 0.3mg zeaxanthin) or 150g corn (0.3mg zeaxanthin and 0.4mg lutein), over a 15-week period that increases in macular pigment density occurred in most subjects within 4 weeks, and this stayed elevated for several months after subjects resumed an unmodified diet.

Johnson et al., (2000)¹⁰⁵ showed similar responses over a 15-week period of supplementation with these foods and also noted significant negative correlations between adipose tissue concentrations and macular pigment for women, but a significant positive relation was found for men.

In a limited study by Landrum et al., (1997)¹⁰⁶ supplementation of lutein esters (equivalent to 30mg unesterified or free lutein/day) over a period of 140 days resulted in a 39 % and 21 % increase respectively in macular pigment optical density in the study's two subjects.

This increase causes a 30 to 40% reduction in blue light reaching the photoreceptors, Bruch's membrane, and the retinal pigment epithelium, the tissues most affected by AMD. Berendschot et al., (2000)¹⁰⁷, demonstrated that supplementation with lutein (10mg free lutein in the form of lutein diesters) for 12 weeks increased the macular pigment density by 4 weeks with a mean linear increase of 5.3% using macular pigment maps and 4.1% with spectral reflective analysis. Schweitzer et al., (2002)¹⁰⁸ also reported that supplementation with 6mg/day lutein over 40 days increased optical density of the macular pigment approximately 20 %. Cardinault et al., (2003)¹⁰⁹ in a very limited 5-week study of lutein supplementation (9mg/day) in young and older subjects, reported that while plasma lutein concentrations increased with supplementation, they did not observe a significant difference in macular pigment optical density. This time period would be too short to observe a significant increase in MPOD (Landrum et al., 1997). In contrast, Bone et al., (2003)¹¹⁰ reported finding a significant correlation between lutein supplementation (2.4-30mg/day) and increases in macular pigment optical density. These series of studies used both unesterified and esterified lutein from marigold extract. Serum lutein concentrations in each subject reached a plateau that was correlated with the dose whereas zeaxanthin was less well absorbed. The rate of increase in macular pigment optical density was correlated with the plateau concentration of the lutein in the serum.

A major intervention trial from the Age-Related Eye Study Research Group (AREDS) was published in 2001, but neither lutein nor zeaxanthin were included due to commercial availability at the time. It is of interest to note that this study reported a nonsignificant decrease in the serum levels of lutein and zeaxanthin in subjects at greatest risk for AMD.

The most significant study to date on lutein for atrophic AMD is the Lutein Antioxidant Supplementation Trial (LAST) reported by Richer et al., (2002)¹¹¹. This study was a prospective 12-month, placebo controlled, double blind study with lutein (10mg) versus lutein/antioxidants (10mg) daily supplementation in 90 male subjects with atrophic AMD. Results demonstrated that supplementation increased macular pigment optical density significantly and some measures of visual function including glare recovery, contrast sensitivity, and distal/near visual acuity, showed statistically significant concurrent improvement.

Table 4. Intervention Trials with dietary modification and/or lutein ester supplements and AMD

Study reference	Study type	Treatment Program	No. of subjects	Duration Follow-up	Age Range Mean age	Outcome
Hammond et al., 1997	Intervention	60g spinach (10.8mg L, 0.3mg Z, 5mg β -carotene) 150g corn (0.3mg Z, 0.4mg L)	13	15 weeks	30-65	Increased macula pigment density in most subjects within 4 weeks
Johnson et al., 2000	Intervention	Same as above	7	15 weeks	33-83	Increased serum and buccal L concentrations, significant sex differences noted with negative correlations between adipose L concentration and macular pigment for women and positive relation for men
Landrum et al., 1997	Intervention	30mg L/day (from lutein ester)	2	140 days		3-4 weeks after supplementation, macular pigment increased in density; 30-40% reduction in blue light reaching vulnerable retinal tissue
Berendschot et al., 2000	Intervention	10mg L/day (from lutein ester)	8	12 weeks	18-50 40.6	Supplementation with lutein increased the density of the macular pigment
Schweitzer et al., 2002	Intervention (German study)	6mg L/day		40 days		Supplementation with lutein increased macular pigment density within 30 days
Cardinault et al., 2003	Intervention	9mg L/day	12	5 weeks	26.9	No increase in macular pigment density
Bone et al., 2003	Intervention	2.4-30mg L/day 30mg Z/day	17 21	120 days- 6 months	67.3 >18	Lutein supplementation increased macular pigment density and serum concentration of lutein

5) Role of Lutein Esters in Reducing the Incidence of Cataract Formation and Extractions

A. Observational and Prospective Studies-Lutein Esters and Cataract

There have been several epidemiological studies conducted on the preventative effects of vitamins on cataracts and these will not be dealt with in this overview except as they may relate to carotenoid data. Appropriate observational studies are summarized in Table 5 and prospective studies in Table 6.

Taylor and Hobbs (2001)¹¹² provided an excellent assessment of nutritional influences on risk for cataract. In an early study linking nutrient intake and cataract extraction by Hankinson et al., (1992)¹¹³, they reported that it was spinach (the leading source of lutein), and not carrots, that was most frequently associated with a lower risk. This observation was followed up in a study involving 77,466 nurses, by Chasan-Taber et al., (1999)¹¹⁴, who found that the risk for cataract extraction was reduced by 22% in those with the highest intake of lutein and zeaxanthin. Brown et al., (1999)¹¹⁵ also showed in a prospective study that men in the highest fifth of lutein and zeaxanthin intake had a 19% lower risk of cataract relative to those in the lowest fifth (RR: 0.8); 95% CI; 0.65, 1.01. Mares-Perlman et al., (1995) found no significant associations between specific carotenoid intake and risk for cataract. This was a nested case-control study within a population based cohort comprised of patients with either retinal pigment abnormalities or late AMD. The authors stated that factors contributing to variability in carotenoid and tocopherol levels, such as using nonfasting serum, may have reduced the statistical power of the analyses. They also stated that mean levels of lutein and zeaxanthin in this study were slightly (but not significantly) lower in cases with advanced AMD and the small number of subjects with exudative AMD may explain a failure to detect relationships with serum carotenoids.

Lyle et al., (1999)¹¹⁶ investigated the relation of serum carotenoids to the incidence of age-related nuclear cataract in 400 adults in the Beaver Dam Eye Study. Although serum carotenoids were not significantly associated with nuclear cataract, a marginal inverse association was reported for lutein (Odd Ratio: 0.3; 95% CI 0.1, 1.2; P=0.13). Berendschot et al., (2002)¹¹⁷ evaluated lens aging in relation to nutrient determinants and possible risk factors for age-related cataracts. They found lens optical density was related to age and macular

pigment optical density. The latter was highly correlated with serum and adipose tissue lutein in men and women. The inverse relationship between macular pigment optical density and lens optical density in the eye is consistent with the hypothesis that lutein and zeaxanthin in the eye retard the effects of aging on the lens.

Jacques et al., (2001)¹¹⁸ found in assessing long-term nutrient intake and early age-related nuclear lens opacities that women with lutein/zeaxanthin intake above 2.4mg/day may have a lower risk of nuclear cataract which supports the findings reported above by Chasan-Taber et al. and Brown et al. In contrast, Gale et al., (1992)¹¹⁹ in earlier work did not observe an association but qualified their findings based on observational data that would need to be confirmed in a randomized controlled trial.

Smoking has been identified to be a risk factor in cataract development. Early work by Christen et al. (1992) provided data supporting the hypothesis that cigarette smoking increases the risk of developing both nuclear sclerosis and posterior subcapsular cataract.

Olmedilla et al. (2001, 2002)^{120 121} reported serum carotenoid concentrations (including lutein and zeaxanthin) in subjects from five European countries as well as for a European Multicenter placebo controlled supplementation study. Their findings show a good correlation with serum carotenoids and eye health and suggest that they can be important markers of a healthy diet. A review of lutein's clinical relevance by Granada et al., (2003) suggests that a serum lutein concentration between 0.6 and 1.05 micromoles/liter seems to be a safe and desirable target associated with a beneficial impact on visual function.

Table 5. Observational studies of lutein (L) and zeaxanthin (Z) and cataract

Study reference	Study type	No. of subjects	Outcome
Taylor and Hobbes, 2001	Observational	NS	Epidemiological studies reviewed relative to associations between antioxidants and reduction in cataract development
Berendschot et al., 2002	Observational Cross/sectional	376	Inverse relationship between lens and macular pigment optical densities suggest L and Z may retard aging of lens
Jacques et al., 2001	Observational/ Cohort	478	Decreasing prevalence of nuclear lens opacities with multivitamin, C and E
Gale et al., 2001	Cross/sectional	372	Risk of posterior subcapsular cataract was lowest in those with higher concentrations of lutein OR=0.5, 95% CI 0.2-1.0, p trend=0.012
Olmedilla et al., 2002	European Multicentral Dietary Supplement	400	Supplementation with lutein from marigold extracts elevated serum lutein 5 fold and zeaxanthin 2 fold
Olmedilla et al., 2001	European Multicentral Dietary Supplement		High concentrations of L and Z from dietary sources increase antioxidant concentrations in plasma and tissues.

Table 6. Prospective Cohort studies investigating the role of dietary carotenoids and age-related cataract development

Study reference	Study type	No. of subjects	Duration Follow-up year	Age Range	Outcome
Hankinson et al., 1992	Prospective Grant Registered Nurses Dietary Questionnaire Biennial	50,828	8	45+	Results showed that spinach (good source of lutein) was consistently associated with lower relative risk of cataract
Brown et al., 1999	Prospective cohort Health Professional Males Dietary Questionnaire	36,644	8	45-75	Men in highest fifth of lutein and zeaxanthin intake had a 19% lower risk of cataract RR=0.81; 95% CI: 0.65-1.01, p<0.03
Chasan-Taber et al., 1999	Prospective cohort Registered Nurses Dietary Questionnaire	77,466	12	45-71	Women with highest intake of L and Z had a 22% decreased risk of cataract extraction
Lyle et al., 1999	Prospective Beaver Dam Eye Study Dietary Questionnaire and Lens Photography for Nuclear opacity	252	18	50-86	Marginal inverse association with lutein OR=0.3; 95% CI: 0.1-1.3, p=0.11 for people >65 yrs age
Christen et al., 1992	Prospective Male Physicians	22,071	12	40-84	Cigarette smoking increases risk for cataract
Schaumberg et al., 2000	Prospective Follow-up Male Physicians	20,271	14	40-84	BMI, height and abdominal adiposity are independent risk factors for cataract

B. Case-Control Studies – Lutein, Zeaxanthin and Cataracts

There are very few case-control studies. These studies are summarized in Table 7. The relationship between cataract and diet was considered in a case-control study conducted in Northern Italy (Tavani et al., 1996)¹²². Findings indicated that diet can play an important role in risk of cataract extraction with a protective action noted for some vegetables, fruit, calcium, folic acid and vitamin E and an increased risk with elevated salt and fat intake.

Leske et al., (1997)¹²³ in a longitudinal study of cataract reported the risk of nuclear opacification was reduced by one-third in regular users of vitamin supplements.

A limited case-control study of nutrition and cataract in low income Mexicans by Sanchez-Castillo et al., (2003)¹²⁴ highlighted the nutritional deficiencies and health problems in Mexico and that intakes of carotenoids from a variety of sources are relatively low (301 μ g/day) and about half of the 500-600 μ g/day WHO reference values.

Olmedilla et al., (2002)¹²⁵ reported in a study of serum status of carotenoids with age-related cataracts, that serum carotenoid levels are highly dependent on dietary intake and thus may not be clinically relevant biomarkers for cataract risk.

Table 7. Case-Control Studies of relationship between lutein and zeaxanthin and age-related cataract development

Study reference	Study type	No. of subjects	Duration Follow-up year	Age Range	Outcome
Tavini et al., 1996	Case-control Patients who had cataract extraction and healthy subjects	207	8	50-74	A significant inverse trend in risk noted for subjects consuming spinach OR=0.6; 95% CI: 0.4-0.9
Leske et al., 1998	Longitudinal Dietary assessment and lens opacities Dietary Questionnaire	764	4.8	40-70(+)	Risk of nuclear opacification was reduced by one third in users of multivitamin supplements RR=0.69; 0.48-0.99
Sanchez-Castillo et al., 2001	Case-control Dietary assessment and lens opacity Exams in low income Mexicans	81		27-67 males 33-79 females	High prevalence of vitamin deficiencies and cataract incidence
Olmedilla et al., 2002	Case-control Patients with uni- or bilateral senile cataracts	138		Median 52 yrs	Visual acuity not related to carotenoids; authors postulate a threshold effect needed

C. Intervention Studies – Lutein, Zeaxanthin and Cataracts

There are two intervention studies with lutein. These are reported in Table 8. Olmedilla et al., (2001)¹²⁶ demonstrated in patients with ARMD and cataracts consuming a 15mg lutein supplement (12 mg all-trans lutein and 3 mg cis-lutein) for 3 days/week for 13-26 months (13 months for ARMD subjects and 26 months for cataract subjects), improvements in visual acuity and glare sensitivity with no significant side effects of hypercarotenemia, carotenodermia or biochemical or hematological effects.

Olmedilla et al., (2003)¹²⁷ also reported that when lutein was supplemented at 15mg/day in a small group of patients with age-related cataracts for 2 years, visual function (visual acuity and glare sensitivity) improved. The author suggested concluded that a higher intake of lutein, through lutein-rich fruit and vegetables or supplements could have beneficial effects on the visual acuity of individuals with age-related cataracts.

Table 8. Intervention Trials with dietary modification and/or lutein esters and age-related cataract

Study reference	Study type	Treatment Regimen	No. of subjects	Duration Follow-up year	Age Range	Outcome
Olmedilla et al., 2001	Intervention Trial	Lutein Esters 12mg lutein/day given 3 times/week Subjects with cataracts or AMD ophthalmic exams	5 5	4-20 months	55-73 69-75	Average improvement in visual acuity of 0.4 and in glare sensitivity
Olmedilla et al., 2003	Intervention 2 yr double-blind study	Lutein Esters 15mg given 3 times/week Patients with age-related cataracts	17	2 yrs	55-73	Visual performance (visual acuity and glare sensitivity) significantly improved at the end of 2 years

6) Conclusions on Available Scientific Data

In summary, it is clear that even with inconsistencies in the epidemiological literature, there is sufficient data to indicate that lutein esters are a dietary source of lutein and zeaxanthin. Further, lutein and zeaxanthin can play both a preventive and a treatment role in age-related macular degeneration and cataract formation. Data demonstrate clearly that macular carotenoids can effectively protect the retina during acute exposure to high light levels from photic-induced oxidative damage. The evidence also supports the hypothesis that the macular pigment can protect the retina from damage due to chronic blue light exposure. Many potential mechanisms plausibly explain how lutein and zeaxanthin's antioxidant function could protect against AMD and age-related cataracts.

While data addressing the question of whether lutein and zeaxanthin are effective in treating early AMD is based on intervention studies whose control can be challenged, the most promising and well controlled study to date is the Lutein Antioxidant Supplementation Trial conducted by Richer et al., (2002)¹¹¹. This was a prospective 12-month, placebo-controlled, double-blind, repeated measures and crossover of lutein vs. lutein/antioxidants on 90 males (74.7 +/- 7.1 years) with atrophic AMD. The results support earlier studies for a significant concurrent

improvement in glare response, contrast sensitivity and distal/near visual acuity in all treatment groups. It appears that some improvements in visual function may result from lutein/zeaxanthin supplementation. The possibility that lutein supplementation may result in a modest delay of the onset of the more severe 'wet form' would be a major contribution to the health of 'at risk' patients and provides further support for the requested qualified health claim that is the subject of this petition.

Additional Issues To Address In Qualified Health Claim Petitions

A. Foods supplemented with lutein esters would provide a meaningful amount of lutein and zeaxanthin in the diet

At present, the optimal level of lutein ester intake has not been defined. Neither FDA nor the IOM Expert Panel of the National Academy of Sciences has established a daily value for lutein. The lutein intakes investigated in the intervention studies discussed above (see Table 4) ranged from 2.4mg to 30 mg/day. The study by Seddon et al.(1994) demonstrated that an intake of 6 mg/day seen in the upper quartile of this study was associated with a significant decrease in the risk of developing AMD. The study reported by Bone et al., (2003) tested the full range of intakes ranging (from 2.4 to 30 mg/day). The results showed that serum lutein levels were influenced at lutein intakes as low as 2.4 mg/day. Macular pigmentation was also affected by supplementation at these low levels. Other studies that used low level lutein intakes (Berendschot et al., 2000, and Schweitzer et al., 2002) also showed increased pigmentation at about 5-6 mg/day.

This supports the conclusion that an intake of 6mg/day lutein would have a positive effect on macular and lens pigment density and would delay AMD and potentially cataract development. The current estimated U. S. intake of 2mg lutein per day from the diet is well below this amount and increased lutein intake is therefore desirable. Additional lutein intake of at least 4 mg/day would provide enough total lutein to have a positive effect on increasing MP and reducing the risk of disease.

The Petitioner recommends that conventional foods be required to contain at least 1.5 mg lutein (3.0 mg lutein esters) per serving in order to be allowed to bear the qualified health claim for lutein and reduced risk of AMD and cataracts. Consistent with requirements for foods eligible to bear several other health claims, this is based on the desirable intake of 6mg/day and 4 eating occasions per day. Dietary supplements should provide at least 6 mg/day(12 mg lutein esters) of lutein. Intake of about 3 mg/serving of Xangold® lutein esters from supplemented foods or 12mg/day from dietary supplements would clearly provide a meaningful amount of additional lutein in the diet while not displacing existing food sources.

B. Public Health Benefit

The public health benefit for the general population from the requested qualified health claim for Zangold® lutein esters is that it would lead to increased consumption of lutein and zeaxanthin, which would be highly desirable for optimal eye health.

The National Eye Institute (NEI) of the National Institutes of Health has issued a statement on their website titled “Lutein and its Role in Eye Disease Prevention” (www.nei.nih.gov/news/statements/lutein.htm)¹²⁸. While pointing out that possible benefits of lutein supplementation for the eye remain uncertain at this time, NEI acknowledges that studies suggest a link between lutein and decreased risk of eye disease.

As part of NEI’s ongoing investigation into the role of nutrition in eye disease, lutein is being evaluated. Specifically, NEI is conducting a lutein absorption study in people over 60 and is also supporting a study that compares the intake of lutein plus zeaxanthin and its role in obviating or lessening the likelihood of developing AMD and/or cataract. This latter study is named the “Carotenoids and Age-Related Eye Disease in Women’s Health Study” and will assist in dietary recommendations by health professionals regarding consumption of diets rich in lutein and zeaxanthin. It will also provide information for future clinical trials on the effectiveness of supplements of lutein and zeaxanthin on the progression of age-related eye disease.

In addition, a two-year randomized controlled trial investigating the effect of a nutritional supplement containing lutein (6mg), vitamins A, C and E, zinc, and copper is underway to

evaluate the effect of the supplement on measures of visual function in people with and without age-related macular disease (Bartlett and Eperjesi, (2003))¹²⁹. This work is being supported by the College of Optometrists in the United Kingdom.

Clearly, these organizations recognize the relationship reported in previous studies showing a positive effect of lutein or lutein esters as a source of lutein on age-related eye disease and are undertaking these studies to both validate early findings and to ultimately use this information to make public health recommendations for eye health.

As a constituent of many foods, lutein esters are considered safe for consumption and their presence in foods has not been reported to cause any adverse health effects. In fact, it has been reported that South Pacific Islanders consume as much as 27mg lutein/day (equivalent to 54 mg lutein esters/day) without reports of adverse events. No significant adverse effects were reported in any of the scientific literature in clinical studies where lutein esters were given for prolonged periods of time at doses up to 60mg/day (equivalent of 30 mg/day lutein). No adverse effects are anticipated from the consumption of Xangold[®] lutein esters as a source of lutein as a supplement alone or added to various food products.

At present, the optimal level of lutein or lutein ester (as a source of lutein) intake has not been defined. Improvements in visual acuity parameters in patients with AMD and cataracts have been reported at doses as low as 2.4-3mg/day in some human clinical studies (Landrum et al., 1997). Other studies of serum adipose and retinal tissues have shown a direct relationship to eye uptake from supplementation with lutein and zeaxanthin. A high dietary lutein intake and increased macular pigment optical density are both inversely related to lens opacity and cataract risk.

Xangold[®] lutein esters are currently ingested at levels of 0.5 to 12mg/day as supplements. Its proposed uses in foods would provide approximately 12mg/day total intake. These levels pose no safety or health concerns and no special considerations are necessary to protect any segment of the population such as children, the elderly, or persons with any known medical condition.

We do not expect any substantial changes in dietary eating habits in the U.S. population from an authorized qualified health claim. No negative consequences would result in the total diet. The beneficial effects of a reduction and/or delay of the onset in two diseases of the eye, namely age-related macular degeneration (AMD) and cataract formation are likely to result from consumption of Xangold® lutein esters in dietary supplements containing 6-12mg/day of lutein esters (equivalent to 3-6 mg unesterified lutein) and in conventional foods supplemented with this ingredient.

7) Summary and Conclusions

As the preceding review demonstrates, the majority of evidence from publicly available scientific studies supports the association of an increased dietary intake of lutein and/or lutein esters with reduction in the risk of age-related macular degeneration and potentially with a reduction in risk of age-related cataract formation. There is also substantial scientific evidence that establishes the biological plausibility and mechanisms for the protective effect by lutein in this role for the prevention or mitigation of the pathophysiological conditions leading to these age-related eye diseases. The scientific literature clearly demonstrates the beneficial physiological and biochemical effects of free lutein, either from dietary lutein or from dietary lutein esters, as a powerful and protective antioxidant. Evidence supports this role for lutein and zeaxanthin in the macula and retina of the eye where lipid and or protein oxidation plays a key role in these age-related diseases. Epidemiological and observational studies in human populations have shown a significant reduction in risk of these eye diseases in individuals with reported high lutein intakes. Furthermore, the majority of case-control, prospective cohort and intervention studies provide evidence for increased macular pigment levels with lutein intake. These data are consistent with a reduction in at risk patients for AMD.

The LAST Study (Lutein Antioxidant Supplementation Trial) provides the most promising evidence to date of lutein's positive role in ameliorating or reducing the risks of age-related macular degeneration. Both the U.S. National Eye Institute of NIH as well as The U.K. College of Optometrists are currently undertaking major clinical trials to elucidate the necessary scientific evidence for lutein's protective action on AMD and cataract in order to make public health recommendations that will benefit the general U.S. and U.K. populations. Age-related macular degeneration is an incurable eye disease that is the leading cause of blindness in those aged 55 and older in the United States, affecting as many as 15 million Americans. As the population ages, we will continue to see the number of cases increase and double over the next 25 years. Cataracts remain the leading cause of low vision, affecting 20.5 million Americans older than 40 years and cataract care consumes 60% of the Medicare budget for vision.

Clearly, the significant public health benefits of increasing lutein consumption are applicable and would render a significant public health benefit to the U.S. population in an

overall reduction in these two diseases of the eye. The current averaged dietary intake of 2mg lutein per day from the diet appears to be well below the target amount of 6mg/day lutein demonstrated to have a positive effect on macular pigment optical density and eye health. It was clearly demonstrated in the work of Seddon et al. (1994) in the upper quintile of intake that a mean intake of 6mg/day of dietary lutein showed a strong statistical reduction in risk of developing ARMD. Dietary levels of approximately 6mg/day lutein are simply not being achieved in the majority of Americans' current diets. Xangold[®] lutein ester supplements and foods containing this product can make a major contribution to eye health by providing convenient sources of lutein and zeaxanthin for consumers.