

The Body of Evidence to Support a Protective Role for Lutein and Zeaxanthin in Delaying Chronic Disease. Overview^{1,2}

Julie A. Mares-Perlman,³ Amy E. Millen, Tara L. Ficek and Susan E. Hankinson*

Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison Medical School, Madison, WI 53705-2397 and *Department of Epidemiology, Harvard School of Public Health, Channing Laboratory Department of Medicine Brigham and Women's Hospital and Harvard Medical School, Boston, MA

ABSTRACT Recent evidence introduces the possibility that lutein and zeaxanthin may protect against the development of the two common eye diseases of aging, cataract and macular degeneration. This potential and the lack of other effective means to slow the progression of macular degeneration have fueled high public interest in the health benefits of lutein and zeaxanthin and the proliferation of supplements containing them on pharmacy shelves. An understanding of the biologic consequences of limiting or supplementing with these carotenoids is only beginning to emerge. Some epidemiologic evidence supports a role in eye disease and, to a lesser extent, cancer and cardiovascular disease. However, the overall body of evidence is insufficient to conclude that increasing levels of lutein and zeaxanthin, specifically, will confer an important health benefit. Future advances in scientific research are required to gain a better understanding of the biologic mechanisms of their possible role in preventing disease. Additional research is also required to understand the effect of their consumption, independent of other nutrients in fruits and vegetables, on human health. The newly advanced ability to measure levels of lutein and zeaxanthin in the retina in vivo creates a unique opportunity to contribute some of this needed evidence. *J. Nutr.* 132: 518S-524S, 2002.

KEY WORDS: • carotenoids • lutein • zeaxanthin • macular degeneration • cataract

Lutein and zeaxanthin belong to the large class of plant pigments referred to as carotenoids. They are more polar than many other carotenoids, due to the presence of hydroxyl groups on the cyclic ring structure. Unlike the provitamin A carotenoids (α - and β -carotene and cryptoxanthin), they cannot be converted to vitamin A. Their presence in tissues is due entirely to ingestion of plant sources; they are not synthesized by animal tissues. However, a variety of metabolites may be found in animal sources, and several exist in human blood and milk (1).

Lutein and zeaxanthin are present in a wide variety of fruits and vegetables (2) and impart a yellow color to the plants they are found in, such as corn. Their concentration is particularly high in leafy green vegetables such as spinach, collards and

kale (2). They are also present in some animal products such as egg yolks (3) due to plant products eaten by animals. American adults, on average, consume ~1–2 mg lutein/d (4). Levels of intake vary considerably across individuals and population subgroups. For example, levels of intake are particularly high among African-Americans, who average 3 mg/day, compared with Caucasians and Hispanic-Americans, who consume one half that level, on average (4). Currently there is speculation that consuming higher levels of these carotenoids leads to higher levels in body tissues, particularly in the eye, and that this may confer health benefits that lower the risk of chronic disease. An overview of current scientific evidence to evaluate this is presented below.

Abundance and distribution in blood and tissues

Clues regarding biologic roles of lutein and zeaxanthin in health and disease may be gained with an understanding of the abundance and distribution in tissues and the variation in abundance across species. Below, the current knowledge of the levels of these carotenoids in blood and tissue is described.

Serum. Lutein and zeaxanthin are major serum carotenoids, along with β -carotene, α -carotene and lycopene (5). Median serum concentrations of lutein and zeaxanthin in American adults, in the Third National Health and Nutrition Examination Survey (NHANES III), ranged from 0.19 $\mu\text{mol/L}$ in the lowest quintile to 0.79 $\mu\text{mol/L}$ in the highest quintile

¹ Presented as part of the symposium "Can Lutein Protect Against Chronic Disease? A Multidisciplinary Approach Involving Basic Science and Epidemiology to Weigh Evidence and Design Analytic Strategies," given at Experimental Biology '01, Orlando, FL, on April 2, 2001. This symposium was sponsored by the American Society for Nutritional Sciences and supported in part by an educational grant from Kemin Foods, Cognis Corporation, United States. Guest editors for the symposium publication were Julie A. Mares-Perlman, University of Wisconsin-Madison, and John W. Erdman, Jr., University of Illinois at Urbana-Champaign.

² Supported by the National Institutes of Health, National Eye Institute grants EY 11722 and EY 13018 the Research to Prevent Blindness.

³ To whom correspondence should be addressed.
E-mail: jmarspe@facstaff.wisc.edu.

(4). Dietary intake of foods rich in these carotenoids has been shown to influence serum concentrations in a positive manner (6–9). However, individual variation in serum response to increased intake has been observed (6,8) and may be due to factors such as varying rates of absorption and tissue uptake. Factors that influence bioavailability are described in detail in a separate manuscript in this issue by Zaripheh and Erdman (10).

Tissue. Lutein and zeaxanthin have also been shown to be major carotenoids found in body tissues (11,12). Levels of these hydroxycarotenoids vary widely across tissues. Reported concentrations of lutein and zeaxanthin are often highest in ocular tissue. In the inner retinal layer, concentrations can range from 0.1 to 1.0 pmol/mm² in tissue (13–15). Mean concentrations in the human cataractous lens are 44.1 (SEM 15.1) ng/g lens wet weight for the epithelial/cortical layer and 15.1 (4.4) ng/g lens wet weight for the nuclear layer (16). Liver lutein/zeaxanthin concentrations are often high, but vary widely from 0 to 16.2 nmol/g (10–13). Other tissues that sometimes have relatively high levels include adrenal, adipose, pancreas, kidney and breast (11,12,17). Levels are often reported to be lower in the lung, spleen, heart, testes, thyroid, ovary and skin (11,12,17,18).

The proportion of total carotenoids in tissue comprised of lutein and zeaxanthin varies as well. Macular pigment in the human retina, unlike other tissues, contains only lutein and zeaxanthin to the exclusion of other carotenoids (19–21), and the lutein/zeaxanthin concentration (1 mmol/L) is ~500-fold higher than the concentration in other tissues (2 μmol/L) (22). Lutein and zeaxanthin represent a large proportion of total carotenoid in some tissues, such as adipose (44%), in which lower proportions of lycopene (39%) and β-carotene (11%) have been reported (11). The proportion of these carotenoids reported in other tissues is minor. For example, in the adrenal, zeaxanthin (with lutein) accounted for ~5% of total carotenoids compared with lycopene (63%) and β-carotene (28%) (11). These disproportionate concentrations of lutein and zeaxanthin among tissues may indicate selective tissue uptake, possibly producing clues to their biological role (11).

There are distinctive patterns in the distribution of lutein and zeaxanthin within tissues. In the macula of the eye, the concentration of these carotenoids is greatest in the center and declines with increasing eccentricity (13–15,23). An average mass (SD) per unit retinal area was reported to be 1.33 (4.3) ng/mm² at the foveal center compared with 0.81 (0.25) ng/mm² with increasing eccentricity of 1.6–2.5 mm (13). Zeaxanthin is more prominent in the inner macula, averaging 60% of the total. As eccentricity increases away from the fovea, lutein becomes the dominant carotenoid. Reported ratios of lutein:zeaxanthin in the peripheral retina have been between 2:1 and 3:1 (13,14). The distribution of lutein and zeaxanthin in the retina suggests a possible role for lutein in protecting the rods that are concentrated in the peripheral retina (24) and for zeaxanthin in protecting the cones that are concentrated in the central retina (13).

Metabolites of lutein and zeaxanthin have been identified in human blood and tissues (1); however, the sources and function are largely unknown. A prevalent isomer in human retina, mesozeaxanthin, may be metabolized from dietary lutein (21) or may be due to its ingestion because this metabolite has been identified in some foodstuffs (25).

Tissue concentrations of lutein and zeaxanthin vary widely across individuals. The greatest abundance of information regarding intraindividual variation in tissue concentrations

exists in eye tissues. In the macula of the eye, Bone et al. (26) observed wide variability in levels of these carotenoids in 112 eyes obtained from autopsy. Median lutein/zeaxanthin concentrations in the central retina in quartile 1 vs. 4 were 2.05 and 9.08 pmol/mm², respectively. In more peripheral areas of the retina, variations in concentrations across individuals also existed, and were inversely related to having a history of macular degeneration, as were levels in the central retina.

The level of variation across individuals may reflect both diet and genetic factors. The premise that diet will influence lutein and zeaxanthin concentrations is supported by several investigators who have reported increases in macular pigment density with feeding of foods (6) or supplements (7,27) rich in these carotenoids. Genetic determination of macular pigment is supported by correlations between macular pigment density and eye color (28) and by the similarities in macular pigment density across some monozygotic twins (29). However, the magnitude of these influences of diet and genetics on tissue levels in the retina is largely unknown.

Possible roles in protection against disease

Types of scientific evidence. A strong body of evidence to support a role of a nutrient or food component in the delay of chronic disease requires scientific data of a variety of different types to establish the protective role “beyond a reasonable doubt.” The overall strength of the body of evidence can be used as a guideline in setting recommendations and against which to weigh the possible costs (financial, health) of consuming higher levels of a nutrient in food or supplements. Some of the nine criteria that were proposed by Hill (30) in the 1960s as guidelines for demonstrating causal relationships of certain exposures to disease are useful in evaluating the strength of evidence that lutein and zeaxanthin protect against disease. The following modifications of Hill’s list are proposed as important criteria to establish strong evidence for a causal role of lutein and zeaxanthin in protection against chronic disease: 1) Biologic plausibility. Evidence is required to support a known biologic mechanism by which lutein and zeaxanthin might protect against disease. Biologic plausibility is stronger with additional experimental evidence for protection demonstrated in animal models or cell culture. 2) Consistency of relationships across human populations and study designs. This lowers the likelihood that observations of protective relationships that are observed reflect biases associated with a particular study design, study sample or confounding factors that were either unmeasured, poorly measured or unknown. 3) Temporality. The evidence for the hypothesized protective effect of a carotenoid against the development of disease is strengthened when levels of the carotenoid in the diet or body are measured before the onset or worsening of disease. Such evidence, provided by prospective studies and clinical trials, rules out the possibility that diets or body levels low in lutein or zeaxanthin are a consequence of the conditions rather than an antecedent event. 4) Strength of the relationship between level of intake and risk for disease. Strong relationships, indicated by large odds ratios or relative risks, like consistent findings, reduce the likelihood that observations are due to unknown or unmeasured factors. Strong relative risks also indicate that the protective benefit is important. 5) Specificity of the relationships observed to lutein and zeaxanthin, rather than other aspects of diet or life style that accompany the intake of foods rich in these carotenoids is challenging to demonstrate. However, such evidence can be determined systematically in large prospective studies. One can rule out

alternate explanations in subgroups that have and do not have diet or life style correlates that might explain a protective effect. Randomized clinical trials can also provide evidence for a specific effect of lutein and zeaxanthin under specific conditions and time periods. Below, the degree to which these criteria are satisfied by the current body of literature are considered in an overview of the scientific evidence that lutein and zeaxanthin may protect against a variety of chronic diseases.

Eye diseases. There is evidence (summarized below) that suggests that lutein and zeaxanthin may reduce risk for developing the two most common eye diseases in older people, i.e., cataract and macular degeneration. There is also the untested possibility that lutein and/or zeaxanthin may slow progression once these conditions are present. In addition, lutein may slow degeneration of vision in patients with retinitis pigmentosa, a heterogeneous group of slow retinal degenerations. However, only preliminary data in a very small number of patients have been published in which lutein slowed vision loss associated with retinitis pigmentosa in one (31) but not another study (32).

The biologic plausibility that lutein and zeaxanthin protect against the development of cataract and macular degeneration is supported by the fact that they have chemical properties that may retard the pathogenic mechanisms that are thought to promote these degenerative conditions. Oxidative stress is high in the eye due to the intense light exposure and the high rate of oxidative metabolism in the retina. It is thought to contribute to age-related damage that may promote cataract (33) and macular degeneration [previously reviewed by Mares-Perlman and Klein (34)]. The antioxidant properties of lutein and zeaxanthin, recently reviewed by Young and Lowe (35), may reduce the degree to which oxidative damage promotes these diseases or may minimize the damage due to oxidative stress by limiting the degree to which oxygen penetrates membranes (36). However, Krinsky (37), in a separate manuscript in this issue, cites the lack of direct evidence for antioxidant protection of these carotenoids *in vivo*. Because these carotenoids absorb blue light, they may reduce photochemical damage that would otherwise occur in the retina when exposed to light of these wavelengths (38). It has been suggested that exposure of the retina to light can promote the development of macular degeneration (39). Light exposure can also increase the production of free radicals in the lens and retina (40). However, epidemiologic data to support a damaging role of light in macular degeneration is inconsistent (41), although this may reflect the difficulty in capturing actual light exposure over many years in people studied to date.

At present, there are very limited data in animals or cell culture that demonstrate a role of lutein and zeaxanthin in protection against eye disease. In addition to being a relatively new field of inquiry, there are few opportunities to investigate the biologic roles of these carotenoids in age-related diseases in animal models. One reason is that only primates have the anatomical feature of a macula, the part of the human retina that is responsible for central vision and fine detail. It is not possible to study the role of carotenoids in macular degeneration in experimental animals such as rodents who do not have a macula. In monkeys fed diets devoid of plant pigments for several years, levels of these pigments in the macula disappear and retinal abnormalities that resemble age-related degenerative changes in humans (drusen accumulation) appear (42). Another potential animal model with which to study the protective effects of lutein and zeaxanthin on the retina is the quail. Quail retina, like the primate macula, is

dominated by cone photoreceptors and concentrates lutein and zeaxanthin. Preliminary studies indicate an inverse correlation between the level of zeaxanthin in quail retina and light-induced retinal cell death (43).

Although many experimental animals do have lenses, permitting study of the influence of diet on cataract, the levels of lutein and zeaxanthin in the lenses of experimental animals is unknown. Because of the large differences in the accumulation of carotenoids across different species, it cannot be assumed that experimental animals accumulate lutein and zeaxanthin in their ocular lenses, like humans. Evidence of the levels of lutein and zeaxanthin in the lenses of experimental animals is needed. Also needed, is evidence that dietary lutein and zeaxanthin influence levels of these carotenoids in lenses of experimental animals or humans.

Epidemiologic studies are required to document the relevance to human disease and to provide insights about the potential importance of lutein and zeaxanthin in reducing risk for these conditions. Some epidemiologic evidence does support a protective role for lutein against the development of macular degeneration, the leading cause of blindness among older people in Western countries. Lower risk for macular degeneration has been associated with the consumption of food sources of these carotenoids (44), with overall level of lutein and zeaxanthin in the diet (4,45) or with higher levels of these carotenoids in the blood (46) or retina (26,47). However, in several studies, these associations were not observed (48–51) or were observed only in population subgroups (4). These inconsistencies may reflect a true lack of protection. Alternatively, they may reflect limitations in the study designs used or limitations of the protection to certain stages of the disease or particular types of people (for example, people who are genetically predisposed.) If consistent relationships in larger studies or certain population subgroups emerge, then clinical trials may be able to add to the strength of the evidence by further defining the specificity of the relationship to lutein and/or zeaxanthin and further refining a time course of a protective influence.

The epidemiologic evidence to support the possibility that lutein and zeaxanthin have an important role in reducing risk of cataract in humans is somewhat consistent. Lower prevalence of nuclear cataract in women (52) or men (43) was associated with intakes of lutein and zeaxanthin in high, compared to low quintiles. Furthermore, in three prospective cohort studies (54–56), people in the highest quintile for the intake of these carotenoids has a 20%–50% lower risk of having cataract extractions (55,56) or of developing nuclear cataract (54). Levels of lutein in blood, although inversely related to the onset of nuclear cataracts in a subgroup of one of the above studies (57) were not significantly related to having nuclear cataracts. However, the statistical power of this observation was limited by the small sample size. Additional epidemiologic studies that demonstrate consistency of associations across populations and further evaluate the strength of the association are warranted. Evidence is also needed to prove that the findings are, specifically, due to the intake of lutein and zeaxanthin, rather than other aspects of diet or life style that are more common in people who eat diets rich in these carotenoids. Thus, there is emerging, but currently insufficient evidence to support the possibility that lutein and zeaxanthin promote eye health.

Lutein and zeaxanthin may play a role in the health of other body tissues, as well. Although these relationships are largely unexplored, there is the possibility that lutein, together with other carotenoids, may protect against cancer, cardiovas-

cular disease and other conditions that may involve the immune system.

Immune function. Research on the immunomodulatory influence of lutein and zeaxanthin suggests that these hydroxycarotenoids may lessen development of other diseases of compromised immunologic status. Whether lutein may enhance monocyte function by increasing the number of surface molecules expressed by monocytes has been investigated (58). Although results of some previous research indicated that β -carotene had such an effect, lutein did not elicit a similar response (58). Other studies support the hypothesized immune-enhancing effects of lutein. Kim et al. (59) showed that supplementation with lutein can elicit an enhancement of an immune response in cats. No epidemiologic studies have yet investigated the relationships between lutein and immunologic markers, (e.g., cytokines, lymphocytes, C reactive protein or neopterin); thus, the potential importance of this possible health benefit is unknown.

Cancer. Carotenoids singly or in combination could lower cancer risk by their ability to scavenge free radicals, to be antimutagenic, to protect against tumor development and to improve immune response. Lutein and β -carotene quench peroxyl radicals and demonstrate antioxidant properties against oxidative damage in vitro (60,61). Plasma lutein analyzed from 37 women correlated inversely with measured oxidative indices (62). It has been shown in vitro, using multilamellar liposomes, that carotenoids in combination elicit a greater antioxidant defense than singularly. The presence of lutein or lycopene produced the strongest synergistic effect (63).

Lutein may be anticarcinogenic as well. This is suggested by its ability to interact with the mutagens 1-nitro pyrene and aflatoxin B₁ (64,65). Lutein may also exert an anticarcinogenic effect by stimulating certain genes involved in T-cell transformations activated by mitogens, cytokines and antigens (66).

Investigation into lutein's protection against site-specific cancers is beginning to evolve in epidemiologic studies and animal models. No associations have been detected between plasma lutein and zeaxanthin concentrations and gastric cancer (67). Slattery et al. (68) detected an inverse association between dietary lutein intake and colon cancer in men and women. The reduction in risk was significant only in those who were diagnosed with colon cancer at a younger age (68). Xanthophyll esters are found in human skin (18). A combination of carotenoids may protect against the development of erythema in human skin (69) and are correlated with the presence or absence of skin cancer and precancerous lesions (68). The specific effects of lutein on skin cancer are yet to be determined.

Past research has shown modest relationships between the consumption of nutrients found in carotenoid rich foods, such as β -carotene and vitamin A, and a reduced risk of breast cancer (71–73). Focus on the potential protective effects of lutein on developing breast cancer has evolved only recently. Recent research in mice showed that low levels of dietary lutein at 0.002 and 0.02% of the diet inhibited mammary tumor incidence, growth and latency (74). Lutein has been shown to induce apoptosis in transformed but not normal human mammary cells and to protect normal cells from apoptosis induced in cell culture (75). Research in population studies is growing. Freudenheim et al. (76) showed that the intake of carotenoid-rich foods, specifically vegetables, as well as lutein and zeaxanthin, is significantly associated with a lower risk for premenopausal breast cancer. In a case-control study, increasing serum levels of lutein and zeaxanthin were

related, in a dose-dependent manner, to the decreased risk of breast cancer, but the trend was only marginally significant (77). A decreased risk of cancer was associated with increasing levels of breast tissue lutein and zeaxanthin concentrations in women with breast cancer compared with women with benign breast biopsies, but the association was not significant (17). In the Nurse's Health Study, Zhang et al. (78) found a weak, but significant, inverse association between lutein and zeaxanthin intake and risk of breast cancer among premenopausal women. The protective effect of lutein and zeaxanthin on breast cancer was strongest among women with a family history of breast cancer. In a nested case-control study from the prospective New York University Women's Health Study, Toniolo et al. (79) found an inverse association between plasma lutein, but not zeaxanthin, and risk of breast cancer. However, plasma α - and β -carotene were also significantly related to a decrease in risk. Other case-control studies have shown no differences in breast adipose tissue concentrations of lutein and zeaxanthin between women with benign breast tumors and those with breast cancer (80).

Despite evidence for specific benefits of lutein and zeaxanthin in protection against cancers, questions remain whether the hypothesized protective effects of serum carotenoids are not a direct result of carotenoid intake but serve rather as a marker for fruit and vegetable intake. Many components of whole fruits and vegetables may be protective, not merely specific carotenoids. In a study by Collins et al. (81), DNA damage was assessed from lymphocytes of volunteers supplemented with β/α -carotene, lutein, lycopene or placebo. Oxidized pyrimidines correlated inversely with total serum carotenoids, lutein and β -carotene in basal measurements. Supplementation with carotenoids did not significantly decrease the measured DNA damage. This supports the notion that some other component of fruits and vegetables may be the effective agent in preventing oxidative damage and that serum carotenoids are merely markers for fruit and vegetable intake. Thus, a specific benefit of lutein and zeaxanthin, independent of other components of fruits and vegetables has not yet been demonstrated in humans. Nevertheless, those carotenoids may be two of the many components of fruits and vegetables that help to protect against cancer.

Heart disease and stroke. Several papers have investigated the potential protective mechanisms of carotenoids, such as lutein, on cardiovascular risk. Lutein and other carotenoids have been shown to act as antioxidants, scavenging peroxynitrite in vitro (82). Martin et al. (83) showed that lutein was effective in reducing the adhesion molecules on the cell surface of confluent human aortic endothelial cells, a necessary modulation of the vasculature in the pathogenesis of atherosclerosis. Lutein inhibited the adhesion of monocytes in one in vitro artery model (84) but not another (83). Lutein supplementation in apo-E-null mice reduced the size of atherosclerotic lesions (84).

Associations have been found between individuals in the highest serum or dietary lutein levels and lower rates of coronary heart disease (82) or stroke (83). In two epidemiological studies, individuals with the highest serum levels of lutein plus zeaxanthin had a significantly reduced risk of coronary heart disease as measured by carotid intima thickness (84,85). Intake of green leafy vegetables was associated with a significant reduction of incident ischemic stroke in the Nurse's Health and Health Professionals Follow-up Study combined (86). Whether these observed associations reflect an independent role for these hydroxycarotenoids in foods or a synergistic role, in combination with the other antioxidants and phytochemi-

cals also found in these foods, is not known. Additionally, it is difficult to distinguish whether low serum carotenoid levels are a cause or risk factor for cardiovascular disease or a reflection of increased lipoprotein density and/or inflammation (87). Thus, the epidemiologic evidence for a protective role of lutein, specifically, is limited.

Summary. Advances in the ability to measure the levels of specific carotenoids in foods and tissues have led to the accumulation of evidence, in the past decade, that lutein and zeaxanthin may promote health and protect against chronic disease. The evidence for a role in eye health is strongest, due to their exclusive presence in ocular tissues and the number of epidemiologic studies that have been conducted to date. Yet, the overall body of evidence is currently insufficient to conclude that a health benefit exists, even in preventing age-related eye disease. There is also the possibility that lutein and zeaxanthin act together with other carotenoids and phytochemicals to promote health of many body tissues. This area of research is only beginning to evolve.

Future directions

We can expect to gain a better understanding of a protective role of lutein and zeaxanthin against chronic disease in the future. One reason is the existence of several ongoing prospective studies in which epidemiologic associations between lutein and zeaxanthin in the diet and the occurrence of disease can be evaluated further. Also, the frequent interchange of information in this field between basic and epidemiologic scientists in jointly attended symposia and in jointly read scientific journals seeds future research advances. Many insights into needs for future research and interpreting the current state of the science were gained in the presentations within this symposium and multidisciplinary discussion that followed.

In this issue, we also gain insights about the ability to quantitate levels of lutein and zeaxanthin in the retina (88). This suggests the possibility that these measurements may be useful in the future as a biomarker of lutein availability. This ability to estimate the quantity of lutein and zeaxanthin in the retina in a noninvasive manner provides a useful tool that is not available in investigating the role of other nutrients against chronic disease. Currently, the validity of this measurement as a biomarker of lutein and zeaxanthin availability is unclear and is being investigated. Observations of increases in macular pigment with the feeding of these carotenoids in foods (6) or supplements (7,27) are supportive of the use of levels in the retina as a biomarker of exposure. However, in the several small studies conducted to date, the relationships between levels in the diet and macula are small and inconsistent (8,29,89). These early results may reflect measurement error in the estimation of dietary lutein. They may also reflect needed improvements in the technique to estimate retinal carotenoid levels by reducing the variability of measurement within individuals and capturing the variability among people.

The usefulness of retinal macular pigment density determinations as a biomarker of lutein and zeaxanthin bioavailability will also be enhanced by the ability to understand, measure and adjust for factors that influence the levels of these carotenoids in the retina. Several studies suggest that smoking (28,89–91), body fat (8) and genetic factors (eye color) (29,89–91) are correlated with the level of macular pigment. Further investigations of these associations, just beginning in larger populations, will provide further insights about the other determinants of retinal lutein and zeaxanthin. These

results can improve our ability to use estimates of carotenoid levels in the retina as a biomarker of exposure. Application of this technology may eventually enhance the degree to which we will be able to progress in understanding relationships of lutein and zeaxanthin to eye disease and, perhaps, to other chronic diseases.

LITERATURE CITED

1. Khachik, F., Bernstein, P. & Garland, D. L. (1997) Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Investig. Ophthalmol. Vis. Sci.* 38: 1802–1811.
2. Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R. & Lanza, E. (1993) Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J. Am. Diet. Assoc.* 93: 284–296.
3. Bailey, C. A. & Chen, B. H. (1989) Chromatographic analyses of xanthophylls in egg yolks from laying hens fed turf Bermuda grass (*Cynodon dactylon*) meal. *J. Food Sci.* 54: 584–586, 592.
4. Mares-Perlman, J. A., Fisher, A., Klein, R., Palta, M., Block, G., Millen, A. E. & Wright, J. D. (2001) Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the Third National Health and Nutrition Examination Survey. *Am. J. Epidemiol.* 153: 424–432.
5. Parker, R. S. (1989) Carotenoids in human blood and tissues. *J. Nutr.* 119: 101–104.
6. Hammond, B. R., Johnson, E. J., Russell, E. J., Krinsky, N. I., Yeum, K. J., Edwards, R. B. & Snodderly, D. M. (1997) Dietary modification of human macular pigment density. *Investig. Ophthalmol. Vis. Sci.* 38: 1795–1801.
7. Landrum, J. T., Bone, R. A., Joa, H., Kilburn, M. D., Moore, L. L. & Sprague, K. E. (1997) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp. Eye Res.* 64: 57–62.
8. Johnson, E. J., Hammond, R. B., Yeum, K.-J., Qin, J., Xiang, D. W., Castaneda, C., Snodderly, M. D. & Russell, R. M. (2000) Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am. J. Clin. Nutr.* 71: 1555–1562.
9. Bone, R. A., Landrum, J. T., Dixon, Z., Chen, Y. M. & Llerena, C. M. (2000) Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Exp. Eye Res.* 71: 239–245.
10. Zaripheh, S. & Erdman, J. W. (2002) Factors that influence the bioavailability of xanthophylls. *J. Nutr.* 132: 531S–534S.
11. Kaplan, L. R., Lau, J. M. & Stein, E. A. (1990) Carotenoid composition, concentrations, and relationships in various human organs. *Clin. Physiol. Biochem.* 8: 1–10.
12. Schmitz, H. H., Poor, C. L., Wellman, R. B. & Erdman, J. W. (1991) Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. *J. Nutr.* 121: 1613–1621.
13. Bone, R. A., Landrum, J. T., Fernandez, L. & Tarsis, S. L. (1988) Analysis of macular pigment by HPLC: retinal distribution and age study. *Investig. Ophthalmol. Vis. Sci.* 29: 843–849.
14. Handelman, G. J., Dratz, E. A., Reay, C. C. & VanKuijk, J. G. (1988) Carotenoids in the human macula and whole retina. *Investig. Ophthalmol. Vis. Sci.* 29: 850–855.
15. Bone, R. A., Landrum, J. T., Friesed, L. M., Gomez, C. M., Kilburn, M. D., Menendez, E., Vidal, I. & Wang, W. (1997) Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp. Eye Res.* 64: 211–218.
16. Yeum, K. J., Shang, F., Schalch, W., Russell, R. M. & Taylor, A. (1999) Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr. Eye Res.* 19: 503–505.
17. Zhang, S., Tang, G., Russell, R. M., Mayzel, K. A., Stampfer, M. J., Willett, W. C. & Hunter, D. J. (1997) Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects. *Am. J. Clin. Nutr.* 66: 626–632.
18. Wingerath, T., Sies, H. & Stahl, W. (1998) Xanthophyll esters in human skin. *Arch. Biochem. Biophys.* 355: 271–274.
19. Snodderly, D. M., Handelman, G. J. & Adler, A. J. (1991) Distribution of individual macular pigment carotenoids in central retina of macaque and squirrel monkeys. *Investig. Ophthalmol. Vis. Sci.* 32: 268–279.
20. Handelman, G. J., Snodderly, D. M., Krinsky, N. I. & Russett, M. D. (1991) Biological control of primate macular pigment. Biochemical and densitometric studies. *Investig. Ophthalmol. Vis. Sci.* 32: 257–267.
21. Bone, R. A., Landrum, J. T., Hime, G. W., Cains, A. & Zamor, J. (1993) Stereochemistry of the macular carotenoids. *Investig. Ophthalmol. Vis. Sci.* 34: 2033–2040.
22. Schmitz, H. H., Poor, C. L., Gugger, E. T. & Erdman, J. W. (1993) Analysis of carotenoids in human and animal tissues. *Methods Enzymol.* 214: 102–116.
23. Bone, R. A. & Landrum, J. T. (1992) Distribution of macular pigment components, zeaxanthin, and lutein, in human retina. *Methods Enzymol.* 213: 360–367.
24. Sommerburg, O., Siems, G. W., Hurst, S. J., Lewis, W. J., Kliger, S. D. & Van Kuijk, J.G.M.F. (1999) Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Curr. Eye Res.* 19: 491–495.
25. Maoka, T., Arai, A., Shimizu, M. & Matsuno, T. (1986) The first isolation

- of enantiomeric and mesozeaxanthin in nature. *Comp. Biochem. Physiol.* 83: 121-124.
26. Bone, R. A., Landrum, J. T., Mayne, S. T., Gomez, C. M., Tibor, S. E. & Twaroska, E. E. (2001) Macular pigment in donor eyes with and without AMD: a case-control study. *Investig. Ophthalmol. Vis. Sci.* 42: 235-240.
27. Berendschot, T.J.M., Goldbohm, A. R., Klopping, W. A., Van de Kraats, J., Van Norel, J. & Van Norren, D. (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Investig. Ophthalmol. Vis. Sci.* 41: 3322-3326.
28. Hammond, B. R., Jr., Curran-Celentano, J. & Judd, S. (1996) Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vis. Res.* 36: 2001-2012.
29. Hammond, B. R., Fuld, K. & Curran-Celentano, J. (1995) Macular pigment density in monozygotic twins. *Investig. Ophthalmol. Vis. Sci.* 36: 2531-2541.
30. Hill, A. (1965) The environment and disease: association or causal? *Proc. R. Soc. Med.* 58: 295-300.
31. Dagnelie, G., Zorge, I. S. & McDonald, T. M. (2000) Lutein improves visual function in some patients with retinal degeneration: a pilot study via the internet. *Optometry* 71: 147-164.
32. Aleman, T. S., Duncan, J. L., Bieber, M. L., De Castro, E., Marks, D. A., Gardner, L. M., Steinberg, J. D., Cideciyan, A. V., Maguire, M. G. & Jacobson, S. G. (2001) Macular pigment and lutein supplementation in retinitis pigmentosa and usher syndrome. *Invest. Ophthalmol. Vis. Sci.* 42(8): 1873-1881.
33. Varma, S. D., Palaniyandi, S., Devamanoharan, A. H. & Ali, B. S. (1999) Oxygen radicals in the pathogenesis of cataracts-possibilities for therapeutic intervention. In: *Nutritional and Environmental Influences on the Eye* (Taylor, A., ed.), pp. 5-24. CRC Press, Boca Raton, FL.
34. Mares-Perlman, J. A. & Klein, R. (1999) Diet and age-related macular degeneration. In: *Nutritional and Environmental Influences on the Eye* (Taylor, A., ed.), pp. 181-214. CRC Press, Boca Raton, FL.
35. Young, A. J. & Lowe, G. M. (2001) Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys.* 385: 20-27.
36. Subczynski, W. K., Markowska, E. & Siewiesiuk, J. (1991) *Biochim. Biophys. Acta* 1068: 68-72.
37. Krinsky, N. I. (2002) Possible biologic mechanisms for a protective role of xanthophylls. *J. Nutr.* 132: 540S-542S.
38. Sliney, D. H., Mueller, H. A. & Ham, W. T. (1976) Retinal sensitivity to damage from short wavelength light. *Nature (Lond.)* 260: 153-155.
39. Borges, J., Li, Z.-Y. & Tso, M.O.M. (1990) Effects of repeated public exposures on the monkey macula. *Arch. Ophthalmol.* 108: 727-733.
40. Dayhaw-Barker, P. (1986) Ocular photosensitization. *Photochem. Photobiol.* 46: 1051-1055.
41. McCarty, C. & Taylor, H. R. (1999) Light and risk for age-related eye diseases. In: *Nutritional and Environmental Influences on the Eye* (Taylor, A., ed.), pp. 135-160. CRC Press, Boca Raton, FL.
42. Malinow, M. R., Feeney-Burns, L., Peterson, L. H., Klein, M. L. & Neuringer, M. (1980) Diet-related macular anomalies in monkeys. *Investig. Ophthalmol. Vis. Sci.* 19: 857-863.
43. Dorey, K., Toyoda, Y., Thomsom, L., Garnett, K. M., Sapunzakis, M., Craft, N., Nichols, C. & Cheng, K. (1997) Light-induced photoreceptor apoptosis is correlated with dietary and retinal levels of 3R,3'R-zeaxanthin. *Investig. Ophthalmol. Vis. Sci. (suppl.)* 38: S355 (Abstract).
44. Goldberg, J., Flowerdew, J., Smith, E., Brody, J. A. & Tso, M.O.M. (1988) Factors associated with age-related macular degeneration: an analysis of data from the First National Health and Nutrition Examination survey. *Am. J. Epidemiol.* 128: 700-710.
45. Seddon, J. M., Ajani, U. A., Sperduto, R. D., Hiller, R., Blair, N., Burton, T. C., Farber, M. D., Gragoudas, E. S., Haller, J., Miller, D. T., Yannuzzi, L. A. & Willett, W. for the Eye Disease Case-Control Study Group (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *Eye Disease Case-Control Study Group. J. Am. Med. Assoc.* 272: 1413-1420.
46. Eye Disease Case-Control Study Group (1992) Risk factors for neovascular age-related macular degeneration. *Arch. Ophthalmol.* 110: 1701-1708.
47. Beatty, S., Murray, I. J., Henson, D. B., Caredo, D., Koh, H. H. & Boulton, M. E. (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Investig. Ophthalmol. Vis. Sci.* 42: 439-446.
48. Mares-Perlman, J. A., Brady, W. E., Klein, R., Klein, B.E.K., Bowen, P., Stacewicz-Sapuntzakis, M. & Palta, M. (1995) Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch. Ophthalmol.* 113: 1518-1523.
49. Mares-Perlman, J. A., Klein, R., Klein, B.E.K., Greger, J. L., Brady, W. E., Palta, M. & Ritter, L. L. (1996) Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch. Ophthalmol.* 114: 991-997.
50. VandenLangenberg, G. M., Mares-Perlman, J. A., Klein, R., Klein, B.E.K., Brady, W. E. & Palta, M. (1998) Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am. J. Epidemiol.* 148: 204-214.
51. Sanders, T.A.B., Haines, A. P., Wormald, R., Wright, L. A. & Obeid, O. (1993) Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. *Am. J. Clin. Nutr.* 57: 428-433.
52. Jacques, P. F., Chylack, L. T., Jr., Hankinson, S. E., Khu, P. M., Rogers, G., Friend, J., Tung, W., Wolfe, J. K., Padhye, N., Willett, W. C. & Taylor, A. (2001) Long-term nutrient intake and early age-related nuclear lens opacities. *Arch. Ophthalmol.* 119(7): 1009-1019.
53. Mares-Perlman, J. A., Brady, W. E., Klein, B. E. K., Klein, R., Haus, G. J., Palta, M., Ritter, L. L. & Shoff, S. M. (1995) Diet and nuclear lens opacities. *Am. J. Epidemiol.* 141 (4): 322-334.
54. Lyle, B. J., Mares-Perlman, J. A., Klein, B.E.K., Klein, R. & Greger, J. L. (1999) Dietary antioxidants and incidence of age-related nuclear cataracts. *Am. J. Epidemiol.* 149: 810-819.
55. Brown, L., Rimm, E. B., Giovannucci, E. L., Chasan-Taber, L., Seddo, J. M., Spiegelman, D., Willett, W. C. & Hankinson, S. E. (1999) A prospective study of carotenoid intake and risk of cataract extraction in U.S. men. *Am. J. Clin. Nutr.* 70: 517-524.
56. Chasan-Taber, L., Willett, W. C., Seddon, J. M., Stampfer, M. J., Rosner, B., Colditz G. A., Speizer, F. E. & Hankinson, S. E. (1999) A prospective study of carotenoid and vitamin A intake and risk of cataract extraction among U.S. women. *Am. J. Clin. Nutr.* 70: 509-516.
57. Lyle, B. J., Mares-Perlman, J. A., Klein, B.E.K., Klein, R., Palta, M., Bowen, P. E. & Greger, J. L. (1999) Serum carotenoids and tocopherols and incidence of age-related nuclear cataract. *Am. J. Clin. Nutr.* 69: 272-277.
58. Hughes, D. A., Wright, A.J.A., Finglas, P. M., Polley, A.C.J., Bailey, A. L., Astley, S. B. & Southon, S. (2000) Effects of lycopene and lutein supplementation on the expression of functionally associated surface molecules on blood monocytes from healthy male nonsmokers. *J. Infect. Dis.* 182: S11-S15.
59. Kim, H. W., Chew, B. P., Wong, T. S., Park, J. S., Weng, B.B.C., Byrne, K. M., Hayek, M. G. & Reinhart, G. A. (2000) Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. *Vet. Immunol. Immunopathol.* 73: 331-341.
60. Iannone, A., Rota, C., Bergamini, S., Tomasi, A. & Canfield, L. M. (1998) Antioxidant activity of carotenoids: an electron-spin resonance study on beta-carotene and lutein interaction with free radicals generated in a chemical system. *J. Biochem. Mol. Toxicol.* 12: 299-304.
61. Sujak, A., Gabrielska, J., Grudziński, W., Borc, R., Mazurek, P. & Gruszecki, W. I. (1999) Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch. Biochem. Biophys.* 371: 301-307.
62. Haeghele, A. D., Gillette, C., O'Neill, C., Wolfe, P., Heimendinger, J., Sedlacek, S. & Thompson, H. J. (2000) Plasma xanthophylls carotenoids correlate inversely with indices of oxidative DNA damage and lipid peroxidation. *Cancer Epidemiol. Biomark. Prev.* 9: 421-425.
63. Stahl, W., Junghans, A., De Boer, B., Driomina, E. S., Briviba, K. & Sies, H. (1998) Carotenoid mixture protect multimellor liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Lett.* 427: 305-308.
64. Gonzales de Mejia, E., Loarca-Pina, G. & Ramox-Gomez, M. (1997) Antimutagenicity of xanthophylls present in Aztec marigold (*Tagetes erecta*) against 1-nitropyrene. *Mutat. Res.* 389: 219-226.
65. Gonzales de Mejia, E., Ramos-Gomez, M. & Loarca-Pina, G. (1997) Antimutagenic activity of natural xanthophylls against aflatoxin B1 in *Salmonella typhimurium*. *Environ. Mol. Mutagen.* 30: 346-353.
66. Park, J. S., Chew, B. P., Wong, T. S., Zhang, J. & Magnuson, N. S. (1999) Dietary lutein but not astaxanthin or beta-carotene increases pim-1 gene expression in murine lymphocytes. *Nutr. Cancer.* 33: 206-212.
67. Tsubono, Y., Tsugane, S. & Gey, K. F. (1999) Plasma antioxidant vitamins and carotenoids in five Japanese populations with varied mortality from gastric cancer. *Nutr. Cancer* 34: 56-61.
68. Slattery, M. L., Benson, J., Curtin, K., Ma, K., Schaeffer, D. & Potte, R. J. D. (2000) Carotenoids and colon cancer. *Am. J. Clin. Nutr.* 71: 575-582.
69. Stahl, W., Heinrich, U., Jungmann, H., Sies, H. & Tronnier, H. (2000) Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *Am. J. Clin. Nutr.* 71: 795-798.
70. Hata, T. R., Scholz, T. A., Ermakov, I. V., McClane, R. W., Khachik, F., Gellermann, W. & Pershing, L. K. (2000) Non-invasive raman spectroscopic detection of carotenoids in human skin. *J. Invest. Dermatol.* 115: 41-48.
71. Hunter, D. J., Manson, J. E., Colditz, G. A., Stampfer, M. J., Rosner, B., Hennekens, C. H., Speizer, F. E. & Willett, W. C. (1993) A prospective study of the intake of vitamins C, E, and A and the risk of breast cancer. *N. Engl. J. Med.* 329: 234-240.
72. Rohan, T. E., McMichael, A. J. & Baghurst, P. A. (1988) A population-based case-control study of diet and breast cancer in Australia. *Am. J. Epidemiol.* 128: 478-489.
73. Potischman, N., McCullosh, E., Byers, T., Nemoto, T., Stubbe, N., Milch, R., Parker, R., Rasmussen, K. M., Root, M., Graham, S. & Campbell, T. C. (1990) Breast cancer and dietary and plasma concentrations of carotenoids and vitamin A. *Am. J. Clin. Nutr.* 52: 909-915.
74. Park, J. S., Chew, B. P. & Wong, T. S. (1998) Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. *J. Nutr.* 128: 1650-1656.
75. Sumantran, V. N., Zhang, R., Lee, D. S. & Wicha, M. S. (2000) Differential regulation of apoptosis in normal versus transformed mammary epithelium and retinoic acid. *Cancer Epidemiol. Biomark. Prev.* 9: 257-263.
76. Freudenheim, J. L., Marshall, J. R., Vena, J. E., Laughlin, R., Brusure, J. R., Swanson, M. K., Nemoto, T. & Graham, S. (1996) Premenopausal breast cancer risk and intake of vegetables, fruits and related nutrients. *J. Natl. Cancer Inst.* 88: 340-348.
77. Dorgan, J. F., Sowell, A., Swanson, C. A., Potischman, N., Miller, R.,

- Schussler, N. & Stephenson, H. E., Jr. (1998) Relationships of serum carotenoids, retinol, α -tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). *Cancer Causes Control* 9: 89–97.
78. Zhang, S., Hunter, D. J., Forman, M. R., Rosner, B. A., Speizer, F. E., Colditz, G. A., Manson, J. E., Hankinson, S. E. & Willett, W. C. (1999) Dietary carotenoids and Vitamins A, C, E and risk of breast cancer. *J. Natl. Cancer Inst.* 91: 547–556.
79. Toniolo, P., Van Kappel, A. L., Akhmedkhanov, A., Ferrari P., Kato, I., Store, R. E. & Riboli, E. (2001) Serum carotenoids and breast cancer. *Am. J. Epidemiol.* 153: 1142–1147.
80. Yeum, K., Ahn, S., Rupp de Paiva, S. A., Lee-Kim, Y. C., Krinsky, N. I. & Russell, R. M. (1998) Correlations between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. *J. Nutr.* 128: 1920–1926.
81. Collins, A. R., Olmedilla, B., Southon, S., Granado, F. & Duthie, S. J. (1998) Serum carotenoids and oxidative DNA damage in human lymphocytes. *Carcinogenesis* 19: 2159–2162.
82. Panasenko, O. M., Sharov, V. S., Briviba, K. & Sies, H. (2000) Interaction of peroxynitrite with carotenoids in human low density lipoproteins. *Arch. Biochem. Biophys.* 373: 302–305.
83. Martin, K. R., Wu, D. & Meydani, M. (2000) The effect of carotenoids on the expression of cell surface adhesion molecules and binding of monocytes to human aortic endothelial cells. *Atherosclerosis* 150: 265–274.
84. Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P., Shircore, A., Hama-Levy, S., Hough, G., Wang, X., Drake, T., Merz, C. N. & Fogelman, A. M. (2001) Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation.* 103 (24): 2922–2927.
85. Iribarren, C., Folsom, A. R., Jacobs, D. R., Gross, M. D., Belcher, J. D. & Eckfeldt, J. H. for the ARIC Study Investigators (1997) Associations of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 17: 1171–1177.
86. Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., Hennekens, C. H., Spiegelman, D. & Willett, W. C. (1999) Fruit and vegetable intake in relation to risk of ischemic stroke. *J. Am. Med. Assoc.* 282: 1233–1239.
87. Kritchvsky, S. B. (1999) β -Carotene, carotenoids and the prevention of coronary heart disease. *J. Nutr.* 129: 5–8.
88. Curran-Celentano, J., Burke, J. D. & Hammond, B. R. (2002) In vivo assessment of retinal carotenoids: macular pigment detection techniques and their impact on monitoring pigment status. *J. Nutr.* 132: 535S–539S.
89. Ciulla, T. A., Curran-Celentano, J. & Cooper, D. (2001) Macular pigment optical density in a Midwestern sample. *Ophthalmology* 108: 730–737.
90. Hammond, B. R., Jr., Curran-Celentano, J., Judd, S., Fuld, K., Krinsky, N. I., Wooten, B. R. & Snodderly, D. M. (1996) Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vis. Res.* 36: 2001–2012.
91. Hammond, B. R. & Caruso-Avery, M. (2000) Macular pigment optical density in a Southwestern sample. *Investig. Ophthalmol. Vis. Sci.* 41: 1492–1497.