Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients

PURPOSE: Dietary carotenoids lutein and zeaxanthin may play a protective role against visual loss from age-related macular degeneration (AMD) through antioxidant and light screening mechanisms. We used a novel noninvasive objective method to quantify lutein and zeaxanthin in the human macula using resonance Raman spectroscopy and compared macular pigment levels in AMD and normal subjects. DESIGN: Observational study of an ophthalmology clinic-based population. PARTICIPANTS AND CONTROLS: Ninety-three AMD eyes from 63 patients and 220 normal eyes from 138 subjects. METHODS: Macular carotenoid levels were quantified by illuminating the macula with a low-power argon laser spot and measuring Raman backscattered light using a spectrograph. This technique is sensitive, specific, and repeatable even in subjects with significant macular pathologic features. MAIN OUTCOME MEASURE: Raman signal intensity at 1525 cm\(^{-1}\) generated by the carbon-carbon double-bond vibrations of lutein and zeaxanthin. RESULTS: Carotenoid Raman signal intensity declined with age in normal eyes (P < 0.001). Average levels of lutein and zeaxanthin were 32% lower in AMD eyes versus normal elderly control eyes as long as the subjects were not consuming high-dose lutein supplements (P = 0.001). Patients who had begun to consume supplements containing high doses of lutein (\(>\approx 4\) mg/day) regularly after their initial diagnosis of AMD had average macular pigment levels that were in the normal range (P = 0.829) and that were significantly higher than in AMD patients not consuming these supplements (P = 0.038). CONCLUSIONS: These findings are consistent with the hypothesis that low levels of lutein and zeaxanthin in the human macula may represent a pathogenic risk factor for the development of AMD. Resonance Raman measurement of macular carotenoid pigments could play an important role in facilitating large-scale prospective clinical studies of lutein and zeaxanthin protection against AMD, and this technology may someday prove useful in the early detection of individuals at risk for visual loss from AMD. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. *Ophthalmology* 2002 Oct;109(10):1780-7.

Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study

We investigated the effect of long-term antioxidant supplementation (lutein and alpha-tocopherol) on serum levels and visual performance in patients with cataracts. Seventeen patients clinically diagnosed with age-related cataracts were randomized in a double-blind study involving dietary supplementation with lutein (15 mg; n = 5), alpha-tocopherol (100 mg; n = 6), or placebo (n = 6), three times a week for up to 2 y. Serum carotenoid and tocopherol concentrations were determined with quality-controlled high-performance liquid chromatography, and visual performance (visual acuity and glare sensitivity) and biochemical and hematologic indexes were monitored every 3 mo throughout the study. Changes in these parameters were assessed by General Linear Model (GLM) repeated measures analysis. Serum concentrations of lutein and alpha-tocopherol increased with supplementation, although statistical significance was reached only in the lutein group. Visual performance (visual acuity and glare sensitivity) improved in the lutein group, whereas there was a trend toward the maintenance of and decrease in visual acuity with alpha-tocopherol and placebo supplementation, respectively. No significant side effects or changes in biochemical or hematologic profiles were observed in any of the subjects during the study. Visual function in patients with age-related cataracts who received the lutein supplements improved, suggesting that a higher intake of lutein, through lutein-rich fruit and vegetables or supplements, may have beneficial effects on the visual performance of people with age-related cataracts. Olmedilla B, Granado F, Blanco I, Vaquero M. *Nutrition* 2003 Jan;19(1):21-4.
Retinal tubulin binds macular carotenoids

PURPOSE: To investigate the biochemical mechanisms responsible for the specific uptake, concentration, and stabilization of the carotenoids lutein and zeaxanthin in the macula. METHODS: Soluble extracts of bovine retina mixed with radioactive carotenoids were purified by hydrophobic interaction, ion exchange, and gel filtration chromatography. Carotenoid-associated proteins in these purified preparations were identified through photoaffinity labeling and protein microsequencing. Similar purifications on human macular tissue without the addition of exogenous carotenoids also were performed. RESULTS: Experiments on bovine retinal tissue demonstrated that tubulin is the major soluble carotenoid-binding protein. When soluble extracts of human macular protein were examined, the endogenous carotenoids lutein and zeaxanthin were found to copurify with tubulin. CONCLUSIONS: Tubulin is found in abundance in the receptor axon layer of the fovea, where it can serve as a locus for the deposition of the high concentrations of macular carotenoids found there. The binding interaction of carotenoids and tubulin in the Henle’s fiber layer could play an important role in the photoprotective effects of the macular carotenoids against the progression of age-related macular degeneration. The association of carotenoids with tubulin, a protein that can form highly ordered linear arrays, may provide an explanation for the dichroic phenomenon of Haidinger's brushes. Bernstein PS, Balashov NA, Tsong ED, Rando RR. Invest Ophthalmol Vis Sci 1997 Jan;38(1):167-75.

Dietary modification of human macular pigment density

PURPOSE: The retinal carotenoids lutein (L) and zeaxanthin (Z) that form the macular pigment (MP) may help to prevent neovascular age-related macular degeneration. The purpose of this study was to determine whether MP density in the retina could be raised by increasing dietary intake of L and Z from foods. METHODS: Macular pigment was measured psychophysically for 13 subjects. Serum concentrations of L, Z, and beta-carotene were measured by high-performance liquid chromatography. Eleven subjects modified their usual daily diets by adding 60 g of spinach (10.8 mg L, 0.3 mg Z, 5 mg beta-carotene) and ten also added 150 g of corn (0.3 mg Z, 0.4 mg L); two other subjects were given only corn. Dietary modification lasted up to 15 weeks. RESULTS: For the subjects fed spinach or spinach and corn, three types of responses to dietary modification were identified: Eight "retinal responders" had increases in serum L (mean, 33%; SD, 22%) and in MP density (mean, 19%; SD, 11%); two "retinal nonresponders" showed substantial increases in serum L (mean, 31%) but not in MP density (mean, -11%); one "serum and retinal nonresponder" showed no changes in serum L, Z, or beta-carotene and no change in MP density. For the two subjects given only corn, serum L changed little (+11%, -6%), but in one subject serum Z increased (70%) and MP density increased (25%). CONCLUSIONS: Increases in MP density were obtained within 4 weeks of dietary modification for most, but not all, subjects. When MP density increased with dietary modification, it remained elevated for at least several months after resuming an unmodified diet. Augmentation of MP for both experimental and clinical investigation appears to be feasible for many persons. Hammond BR Jr, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, Snodderly DM. Invest Ophthalmol Vis Sci 1997 Aug;38(9):1795-801.

Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin

PURPOSE: Although oxidative stress may play an important role in the development of age-related cataract, the degree of protection reported for antioxidant vitamins and carotenoids has been inconsistent across studies. These varied results may be due in part to the lack of good biomarkers for measuring the long-term nutritional status of the eye. The present experiments investigated the relationship between retinal carotenoids (i.e., macular pigment), used as a long-term measure of tissue carotenoids, and lens optical density, used as an indicator of lens health. METHODS: Macular pigment (460 nm) and lens (440, 500, and 550 nm) optical density were measured psychophysically in the same individuals. Groups of younger subjects-7 females (ages 24 to 36 years), and 5 males (ages 24 to 31 years)--were compared with older subjects--23 older females (ages 55 to 78 years), and 16 older males (ages 48 to 82 years). RESULTS: Lens density (440 nm) increased as a function of age (r = 0.65, p < 0.001), as expected. For the oldest group, a significant inverse relationship (y = 1.53-0.83x, r = -0.47, p < 0.001) was found between macular pigment density (440 nm) and lens density (440 nm). No relationship was found for the youngest group (p < 0.42). CONCLUSIONS: The main finding of this study was an age-dependent, inverse relationship between macular pigment density and lens density. Macular pigment is composed of lutein and zeaxanthin, the only two carotenoids that have been identified in the human lens. Thus, an inverse relationship between these two variables suggests that lutein and zeaxanthin, or other dietary factors with which they are correlated, may retard age-related increases in lens density. Hammond BR Jr, Wooten BR, Snodderly DM. Optom Vis Sci 1997 Jul;74(7):499-504.
Identification of lutein and zeaxanthin oxidation products in human and monkey retinas

PURPOSE: To characterize fully all the major and minor carotenoids and their metabolites in human retina and probe for the presence of the oxidative metabolites of lutein and zeaxanthin. METHODS: Carotenoids of a composite of 58 pairs of human retinas and a monkey retina were elucidated by comparing their high-performance liquid chromatography (HPLC)-ultraviolet/visible absorption spectrophotometry (UV/Vis)-mass spectrometry (MS) profile with those of authentic standards prepared by organic synthesis. RESULTS: In addition to lutein and zeaxanthin, several oxidation products of these compounds were present in the extracts from human retina. A major carotenoid resulting from direct oxidation of lutein was identified as 3-hydroxy-beta, epsilon-caroten-3'-one. Minor carotenoids were identified as: 3'-epilutein, epsilon, epsilon-carotene-3,3'-diol, epsilon, epsilon-carotene-3,3'-dione, 3'-hydroxy-epsilon, epsilon-caroten-3-one, and 2,6-cyclolycopene-1,5-diol. Several of the geometric isomers of lutein and zeaxanthin were also detected at low concentrations. These were as follows: 9-cis-lutein, 9'-cis-lutein, 13-cis-lutein, 13'-cis-lutein, 9-cis-zeaxanthin, and 13-cis-zeaxanthin. Similar results were also obtained from HPLC analysis of a freshly dissected monkey retina. CONCLUSIONS: Lutein, zeaxanthin, 3'-epilutein, and 3-hydroxy-beta, epsilon-caroten-3'-one in human retina may be interconverted through a series of oxidation-reduction reactions similar to our earlier proposed metabolic transformation of these compounds in humans. The presence of the direct oxidation product of lutein and 3'-epilutein (metabolite of lutein and zeaxanthin) in human retina suggests that lutein and zeaxanthin may act as antioxidants to protect the macula against short-wavelength visible light. The proposed oxidative-reductive pathways for lutein and zeaxanthin in human retina, may therefore play an important role in prevention of age-related macular degeneration and cataracts. Khachik F, Bernstein PS, Garland DL. Invest Ophthalmol Vis Sci 1997 Aug;38(9):1802-11.

A one year study of the macular pigment: the effect of 140 days of a lutein supplement

A low density of macular pigment may represent a risk factor for age-related macular degeneration (AMD) by permitting greater blue light damage. This study was carried out to determine the effects on macular pigment optical density of dietary supplementation with lutein, one of the pigment constituents. Two subjects consumed lutein esters, equivalent to 30 mg of free lutein per day, for a period of 140 days. Macular pigment optical density was determined by heterochromatic flicker photometry before, during, and after the supplementation period. Serum lutein concentration was also obtained through the analysis of blood samples by high-performance liquid chromatography. Twenty to 40 days after the subjects commenced taking the lutein supplement, their macular pigment optical density began to increase uniformly at an average rate of 1.13+/0.12 milliabsorbance units/day. During this same period, the serum concentration of lutein increased roughly tenfold, approaching a steady state plateau. The optical density curve eventually levelled off 40 to 50 days after the subjects discontinued the supplement. During the same 40 to 50 days, the serum concentration returned to baseline. Thereafter, little or no decrease in optical density was observed. The mean increases in the macular pigment optical density were 39% and 21% in the eyes of the two subjects respectively. In conclusion, the modest period of supplementation has been estimated to have produced in the subjects a 30 to 40% reduction in blue light reaching the photoreceptors, Bruch’s membrane, and the retinal pigment epithelium, the vulnerable tissues affected by AMD. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. Exp Eye Res 1997 Jul;65(1):57-62.