Measurement of Carotenoids, Retinoids, and Tocopherols in Human Lenses

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Purpose. To determine the levels of carotenoids, retinoids, and tocopherols in normal and cataractous human lenses.

Methods. Concentrations of carotenoids, retinoids, and tocopherols were measured by high-performance liquid chromatography (HPLC) in 12 American normal lenses, 9 American cataractous lenses, and 10 Indian cataractous lenses.

Results. On a per gram wet weight of tissue basis, human lenses contained 11 to 25 ng xanthophylls (lutein and zeaxanthin), 31 to 50 ng retinol, 21 to 25 ng retinyl palmitate, 1573 to 2550 ng α-tocopherol, and 257 to 501 ng γ-tocopherol. Concentrations of lutein, zeaxanthin, and retinol were significantly higher in Indian cataractous lenses than in American normal or cataractous lenses. There were no differences in the lutein–zeaxanthin, retinoid, or α-tocopherol contents between American normal lenses and American cataractous lenses. The range of ratios of lutein to zeaxanthin in human lenses was 1.6 to 2.2. The mean age of the Indian lens donors was 20 years younger than the American lens donors. Comparisons using contralateral lenses indicated that there was significant interindividual variance in human lens concentrations of xanthophylls, retinoids, and tocopherols. β-carotene and lycopene, major carotenoids in human serum and other tissues, were not detected in human lenses.

Conclusions. Xanthophylls (lutein and zeaxanthin) are the only carotenoids detected in human lens. Retinol, retinyl palmitate, and α- and γ-tocopherols also are present in human lens. Determinants of lens concentration of nutrients are not defined, but dietary factors are likely to be important. Invest Ophthalmol Vis Sci. 1995;36:2756–2761.

Oxidative damage to the lens has been recognized as a primary event in the pathogenesis of many forms of cataracts.[1-4] It has been reported that elevated plasma or nutrient intake levels of antioxidant vitamins, such as carotenoids, vitamin E, and ascorbic acid, are associated with diminished risk for cataract, and, further, that subjects consuming low amounts of fruits and vegetables are at increased risk for cataract formation.[4-16] Nevertheless, the association between risk for cataract and specific antioxidant nutrients remains controversial. In an 8-year prospective cohort study, Hankinson et al[6] reported that an elevated intake of spinach, which is high in lutein and zeaxanthin (but low in β-carotene content) was most consistently associated with a lower risk for cataract extraction, whereas high β-carotene and vitamin E intakes showed no beneficial effect against cataract. This study corroborated data from Jacques et al,[7] who showed that persons with slightly elevated levels of plasma total carotenoids had a 25% lower risk for any type of cataract. They also showed that consumption of foods rich in carotenoids, other than β-carotene, was associated with a decreased risk for cataract.[8]

Because carotenoids can be effective lipophilic antioxidants, particularly at low partial pressures of oxygen[1,2,3,5] such as in the lens, and because there appears to be a possibility to exploit dietary carotenoids to delay cataract formation, it seemed essential to determine levels of carotenoids, retinoids, tocopherols, and other fat-soluble antioxidant nutrients in normal and

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cataractous lenses. Of these lipophilic antioxidants, data exist only regarding tocopherol levels in lenses of animals.[17,18]

MATERIALS AND METHODS

Chemical Products

All-*trans*-β-carotene (type IV), all-*trans*-retinol, retinyl palmitate, γ-tocopherol, and α-tocopherol were purchased from Sigma Chemical (St. Louis, MO). Lutein was purchased from Kemin Industries (Des Moines, IA). Zeaxanthin, tocol, and γ-carotene were gifts from Hoffmann–La Roche (Nutley, NJ). Solutions of carotenoids and retinoids were prepared under red light immediately before use. All high-performance liquid chromatography (HPLC) solvents were obtained from J. T. Baker Chemical (Philipsburg, NJ) and were filtered through a 0.45-μm membrane filter before use.

Source and Preparation of Tissues

The American normal lenses were obtained frozen from the National Disease Research Interchange (Philadelphia, PA). Lenses were designated as normal if examining physicians or National Disease Research Interchange staff did not indicate the presence of cataracts in the lens and if, on enucleation, no cataracts were observed. The American cataractous lenses were obtained by intracapsular extraction at the Venice Eye Clinic and were kindly provided by Dr. S. Zigman. Lenses from India were provided by the Cooperative Cataract Research Group and were shipped on dry ice and stored at −80°C. Tenets of the Declaration of Helsinki were followed, informed consent was obtained, and institutional human experimentation committee approval was granted. The characteristics of the subjects are listed in Table 1. We were unable to obtain noncataractous lenses from Indians.

The lens tissue was extracted without saponification using $CHCl_3$:CH_3OH (2:1, vol/vol) followed by hexane. Gamma-carotene, retinyl acetate, and tocol were added as internal standards for the analysis of carotenoids, retinoids, and tocopherols, respectively. The mixture was sonicated (model W-800; Heat Systems–Ultrasonics, Farmingdale, NY) twice on ice for 30 seconds and centrifuged for 10 minutes at 800*g* at 4°C. No particulate matter remained after sonication. The chloroform and hexane layers were evaporated to dryness under N_2, and the residue was redissolved in 150 μl ethanol. A 50-μl aliquot was injected onto the HPLC system.

High-Performance Liquid Chromatography Procedures

Carotenoid, Retinoid, and Tocopherol Analysis. The HPLC system consisted of a Series 410 LC pump (Perkin–Elmer, Norwalk, CT), a Waters 717 plus autosampler (Millipore, Milford, MA), a Pecosphere-3 C18 0.46- × 8.3-cm cartridge column (Perkin–Elmer), a Waters 994 programmable photodiode array detector, and a Waters 840 digital 350 data station. The HPLC mobile phase was CH_3CN:THF:H_2O (50:20:30, vol/vol/vol, 1% ammonium acetate in H_2O, solvent A) and CH_3CN:THF:H_2O (50:44:6, vol/vol/vol, 1% ammonium acetate in H_2O, solvent B). The gradient procedure at a flow rate of 1 ml/minute was as follows: 100% solvent A was used for 3 minutes, followed by a 13-minute linear gradient to 100% solvent B, a 13-minute hold at 100% solvent B, then a 2-minute gradient back to 100% solvent A. The Waters 994 programmable photodiode array detector was set at 340 nm for retinoids and 450 nm for carotenoids. A fluorescence detector (Ex:292 nm, Em:330 nm; Waters 470; Millipore) was connected for tocopherol analysis. Using this method, lutein and zeaxanthin coelute, but other major carotenoids are separated adequately. Carotenoids, retinoids, and tocopherols were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. Levels were corrected for extraction and handling losses by monitoring the recovery of the internal standards. The lower limit of detection was 0.2 pmol for carotenoids and 2.0 pmol for retinoids. To assess the interindividual variance in human lens concentrations of carotenoids, retinoids, and tocopherols, as well as intraindividual variance, paired lenses from the same individual in the normal American group were measured for these components. The average values are presented.

Separation of Lutein and Zeaxanthin. Separation of the zeaxanthin and lutein stereoisomers was achieved isocratically with the mobile phase consisting of 83% of solvent A (methanol:methyl-tert-butyl ether:water, 81:15:4, vol/vol/vol) and 17% of solvent B (methanol:methyl-tert-butyl ether:water, 6:90:4, vol/vol/vol) at a flow rate of 1 ml/minute at 20°C on a C30 carotenoid column (3 μm, 150 × 4.6 mm; YMC, Wilmington, NC).

Statistical Analysis

Results are expressed as means ± SEM, and the significance of differences was determined by analysis of variance using the Statview II program (Abacus Concepts, Berkeley, CA).

RESULTS

Measurement of Carotenoids, Retinoids, and Tocopherols in Human Lens

The origin of the lens samples is indicated in Table 1. It is of interest that the mean age in the Indian and American cataract lens groups differs by 20 years. This

TABLE 1. Endogenous Concentrations (ng/g wet wt) of Carotenoids, Retinoids, and Tocopherols in Human Lenses With and Without Cataracts

	Groups		
Age, Sex, Nutrient	*American Normal*	*American Cataract*	*Indian Cataract*
Age*	53.3 ± 6.9	78.6 ± 2.3	58.2 ± 1.4
Male (female)	2 (4)	7 (2)	5 (7)
Lut/zeax	13.8 ± 0.9	11.8 ± 1.4	25.8 ± 3.5†‡
β-carotene	ND	ND	ND
Lycopene	ND	ND	ND
Retinol	38.1 ± 4.2	31.3 ± 3.9	50.4 ± 6.3‡
Retinyl ester	25.6 ± 7.1	25.7 ± 4.9	21.7 ± 3.1
α-tocopherol	1573 ± 168	2126 ± 209	2550 ± 203†
γ-tocopherol	367 ± 64	501 ± 49§	257 ± 24‡

ND = not detected (<0.1 ng).
Values are mean ± SEM.
Analysis of variance comparison procedure was used for statistical analyses of the data. $P < 0.05$.
* Mean donor age ± SEM.
† American normal versus Indian cataract.
‡ American cataract versus Indian cataract.
§ American normal versus American cataract.

is consistent with previous data showing that Indians have fourfold to fivefold the risk for cataract at younger ages. The lenses contained substantial amounts of xanthophylls (lutein and zeaxanthin); however, β-carotene and lycopene, which are major carotenoids in human serum, were not present in human lens tissue (Table 1). The endogenous concentrations of lutein–zeaxanthin in American normal (13.8 ± 0.9 ng/g wet wt tissue) and cataractous lens (11.8 ± 1.4 ng/g wet wt tissue) were not significantly different. However, levels of lutein–zeaxanthin in Indian lenses were significantly higher (25.8 ± 3.5 ng/g wet wt tissue; $P < 0.05$) than either the American normal or cataractous lenses. Examinations of individual lenses (Table 2) indicate that there were considerable interindividual and intraindividual variabilities between contralateral lenses for all measured components.

Other lipid-soluble vitamins, such as retinol, retinyl palmitate, γ-tocopherol, and α-tocopherol, also were detected in human lens (Table 1). Mean levels of retinol were not statistically different between American normal lenses (38.1 ± 4.2 ng/g wet wt tissue) and American cataractous lenses (31.3 ± 3.9 ng/g wet wt tissue), but they were significantly lower ($P < 0.05$) than the levels of retinol in Indian lenses (50.4 ± 6.3 ng/g wet wt tissue). In contrast, lens concentrations of retinyl esters showed no significant difference among the three groups.

The endogenous concentrations of α-tocopherol were significantly lower ($P < 0.05$) in American normal lenses than in American cataractous lenses. Similarly, the levels of α-tocopherol in Indian cataractous lenses were significantly higher ($P < 0.05$) than the levels of α-tocopherol in American normal lenses. However, the reverse pattern was observed for γ-tocopherol. Gamma-tocopherol content was lowest in

TABLE 2. Xanthophyll, Retinoid, and Tocopherol Contents (ng/g wet wt) in Paired American Normal Lenses From the Same Donor

		Lut/Zeax		*Retinol*		*Retinyl Ester*		*α-Tocopherol*		*γ-Tocopherol*	
Age	*Sex*	*Lens 1*	*Lens 2*	*Lens 1*	*Lens 2*	*Lens 1*	*Lens 2*	*Lens 1*	*Lens 2*	*Lens 1*	*Lens 2*
25	F	19.7	15.6	13.7	9.5	92.9	ND	1115	936	288	147
44	F	10.8	9.1	39.0	44.6	28.2	26.5	861	1221	269	298
55	M	13.1	12.7	44.7	60.9	6.5	27.4	1078	1166	681	709
58	M	14.3	15.5	34.6	38.3	17.5	18.7	1919	1545	239	250
66	F	8.2	15.3	40.7	39.8	10.7	23.9	2130	2430	184	114
72	F	15.6	15.8	54.4	36.4	15.7	13.4	2385	2094	644	587

Lut/Zeax = lutein/zeaxanthin; ND = not detected; F = female; M = male.

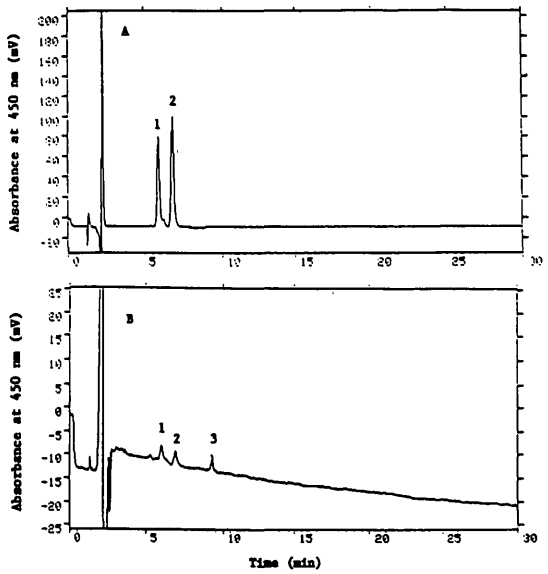

FIGURE 1. High-performance liquid chromatograph of xanthophylls using (**A**) authentic standards and (**B**) human lens. Peak identification: 1 = lutein; 2 = zeaxanthin; 3 = unknown

Indian lenses. It is tempting to speculate that there are age- or cataract-related differences in some values (Table 1). However the data in Tables 1 and 2 do not support this conclusion.

Separation of Lutein and Zeaxanthin in Human Lens

The xanthophylls of human lens were identified by retention times on a C 30 column and absorption maxima equivalent to authentic standards. These were monitored at 450 nm as shown in Figure 1. Peaks 1 and 2 from Figure 1 showed absorption maxima at 445 and 449 nm, respectively (Fig. 2). The retention times and spectra matched authentic standards of lutein and zeaxanthin. The ratio of lutein to zeaxanthin in human lens was 1.6 to 2.2. An unidentified peak, which eluted at 7.5 minutes on the C 30 column, did not have a spectrum that resembled the spectra of stereoisomers of lutein or zeaxanthin.

DISCUSSION

Recent laboratory, clinical, and epidemiologic studies indicate that elevations in lipid antioxidant status is associated with prolonged lens function.[1-3,5-16] To explore these data fully, it is essential to define concentrations of these components in the lens, particularly in response to diet. In this work, we define for the first time human lens concentrations of fat-soluble nutrients lutein–zeaxanthin, retinol, retinyl ester, α-tocopherol, and γ-tocopherol. No β-carotene was detected.

Humans accumulate hydrocarbon carotenoids (such as β-carotene) and xanthophylls (such as lutein and zeaxanthin) in plasma and tissues,[19] but the distribution of each carotenoid is different among various organs.[20] In contrast to β-carotene, xanthophylls, which are widely distributed in nature, possess no provitamin A activity. Xanthophylls are known to act as chain-breaking antioxidants in biologic systems by trapping chain-carrying peroxyl radicals.[21-23] Also, xanthophylls are likely to eliminate the phototoxic blue light selectively.[24]

Several studies have shown that there is a strong inverse association between elevated consumption of dark green vegetables, which are rich in lutein and zeaxanthin, and a decreased risk for oxidative stress-related diseases such as cataract[6,8] and cancer.[25,26] Lutein and zeaxanthin are the only carotenoids that have been reported to be present in several sites of the human eye, such as the retina and macula.[24,27-29] This study shows that lutein and zeaxanthin are also the only carotenoids present in human lens. Human lens contains relatively small amounts of xanthophylls (10 to 20 ng/g wet wt tissue) compared to other organs, such as lung and colon tissues, which contain 80 to 90 ng/g wet wt tissue.[30] Whereas lycopene and β-carotene are the major carotenoids in other organs, these appear to be absent in the human lenses. Although Knekt et al,[11] in a case-control study, indicated that low serum concentrations of β-carotene are a risk fac-

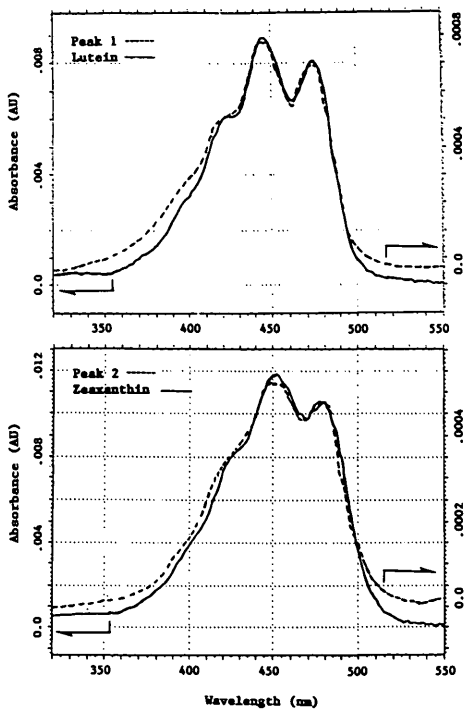

FIGURE 2. The spectra of authentic standards of lutein and zeaxanthin (*solid line*) and the spectra of peaks 1 and 2 from Figure 1 (*dashed line*).

tor for senile cataract, it is probable that in that study, the serum β-carotene really reflects lutein–zeaxanthin status or total carotenoid status. The data by Knekt et al contrast with data from an 8-year prospective study showing that the consumption of carrots, which are rich in β-carotene but not in xanthophylls, was not related to cataract extraction incidence. On the other hand, consumption of spinach, which is rich in lutein and zeaxanthin, showed a consistent inverse relationship with risk of cataract.[6] It is likely that xanthophylls share the free-radical quenching, antioxidant property of other antioxidant nutrients.

The range of ratios of lutein to zeaxanthin in human normal lens was 1.6 to 2.2. This proportion of lutein and zeaxanthin is similar to the human retina ratio of lutein and zeaxanthin.[24] On the other hand, Bone et al[29] observed approximately twice as much zeaxanthin as lutein in the human retina. Another study by Bone et al[31] indicated that major components of the human macular pigments were lutein, zeaxanthin, and meso-zeaxanthin. In our system, the zeaxanthin and meso-zeaxanthin stereoisomers coelute.

In our study, the variance in the data was large, making interpretation difficult. Intrasubject variance is consistent with psychophysical measurements[29,32–34] that found the density of the macular pigment in different normal subjects to range between 0.2 to 1.2 absorbance units. Dietary and supplement usage information was not available on our subjects. It is possible that consumption of xanthophylls, retinoids, and tocopherols influence levels of these components in lens and can, therefore, explain the wide range of values. The high values seen in Indian cataractous lenses appear to be consistent with the vegetarian customs of that society with a high xanthophyll intake. In future studies, it would be useful to compare levels of these nutrients in Indian normal and Indian cataractous lenses and to determine the extent to which age is related to lenticular carotenoid levels.

Determinants of lens concentration of nutrients are not certain but include dietary factors as well as efficiency of nutrient absorption, rate of tissue uptake, and rate of metabolism. Also, it is possible that the various types of cataracts may have different risk factors. Because the concentrations of most of the measured nutrients in Indian cataractous lenses were higher than the levels of American cataractous and normal lenses, it is probable that lens levels of these nutrients are not exclusive determinants of risk for cataract. These data provide the first report regarding human lens levels of carotenoids, retinoids, and tocopherols. The data now make it possible to pursue correlations regarding dietary intake, blood levels, and lens levels of these nutrients. Such data are essential to interpret epidemiologic information indicating that carotenoid intake is related to the risk for cataract and to pursue future studies in which optimal dietary and lenticular carotenoid levels can be defined.

Key Words

aging, carotene, carotenoids, cataract, lens pigment, lutein, tocopherol, zeaxanthin

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