

# Lens Aging in Relation to Nutritional Determinants and Possible Risk Factors for Age-Related Cataract

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**Objective:** To investigate whether nutritional factors and possible risk factors for cataract influence the lens optical density (LOD).

**Design:** Three hundred seventy-six subjects, aged 18 to 75 years, were recruited. In a cross-sectional design, serum was analyzed for lutein, zeaxanthin, vitamin C,  $\alpha$ -tocopherol, and cholesterol levels. Adipose tissue (n=187) was analyzed for lutein level. The LOD and the macular pigment optical density (MPOD) were measured by spectral fundus reflectance.

**Results:** The mean  $\pm$  SD LOD at 420 nm was  $0.52 \pm 0.17$ . It showed a significant association with age ( $\beta = .008$ ,  $P < .001$ ) and MPOD ( $\beta = -.096$ ,  $P = .02$ ). For subjects 50 years and younger (mean  $\pm$  SD LOD,  $0.45 \pm 0.11$ ), we found

only a single significant  $\beta$  coefficient, for age ( $\beta = .006$ ,  $P < .001$ ). For subjects older than 50 years (mean  $\pm$  SD LOD,  $0.68 \pm 0.16$ ), we found significant  $\beta$  coefficients for age ( $\beta = .011$ ,  $P < .001$ ) and MPOD ( $\beta = -.240$ ,  $P = .005$ ). Controlling for age, we found no associations between LOD and other possible risk factors for age-related cataract or serum or adipose tissue concentrations of carotenoids, vitamin C, and  $\alpha$ -tocopherol.

**Conclusions:** Macular pigment is composed of lutein and zeaxanthin, the only carotenoids found in human lenses. The inverse relationship between LOD and MPOD suggests that lutein and zeaxanthin may retard aging of the lens.

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**A**GE-RELATED CATARACT is the leading cause of visual disability.<sup>1</sup> Several nutritional determinants possibly influence age-related cataract. Vitamins C and E and the carotenoids lutein and zeaxanthin are present in human lenses, with vitamin C at concentrations severalfold that of plasma.<sup>2-4</sup> They all can reduce oxidative stress, which influences the development of cataract.<sup>5-7</sup> Low vitamin C content in the lens seemed to be a good indicator of cataract severity.<sup>8</sup>

Plasma levels of vitamin C were not associated with risk of cataract.<sup>9-11</sup> Studies<sup>9-12</sup> on a possible association between plasma levels of vitamin E and risk of cataract showed conflicting results. Self-reported consumption of vitamin C and E supplements was associated with a reduced risk of cataract.<sup>13-16</sup> However, studies<sup>17-21</sup> on consumption of vitamin C supplements showed controversial results. Conflicting results were also reported for multivitamin or vitamin E use.<sup>22-24</sup>

Intake of foods rich in lutein and zeaxanthin, like spinach and broccoli, was as-

sociated with a lower relative risk of cataract.<sup>12,17,25-29</sup> Lutein and zeaxanthin are also components of macular pigment in the eye.<sup>30,31</sup> A significant association has been found between macular pigment optical density (MPOD) and concentrations of serum and adipose tissue lutein,<sup>32,33</sup> be it that there are significant differences between men and women.<sup>34,35</sup> Furthermore, intake of lutein modifies the MPOD.<sup>32,36,37</sup> The MPOD might be a better measure of the lutein and zeaxanthin content in the eye than serum and adipose tissue values of carotenoids, although there is no independent evidence to support this hypothesis.

Besides nutritional determinants, other factors might influence cataract. Smoking has been identified to increase cataract risk.<sup>16,22,38-43</sup> However, smoking is also associated with diminished carotenoid<sup>44</sup> and vitamin C<sup>45</sup> status, and there seemed to be a diminished risk for cataract in smokers who used multivitamins compared with smokers not using multivitamins.<sup>46</sup> Height and abdominal adiposity are independent risk factors for cataract.<sup>47,48</sup> The association between body

mass index (BMI) and cataract is controversial.<sup>22,49-54</sup> Subjects with darker-colored irises tend to have a higher risk of cataract.<sup>55-57</sup> Finally, women have a somewhat higher incidence of cataract than men.<sup>58-61</sup>

There is a strong relation between lens aging and cataract, the latter being the end stage of deterioration of the aging lens.<sup>62-64</sup> Lens senescence can be quantified by the increase in lens optical density (LOD), which has been studied extensively.<sup>62,65-70</sup> Although these studies showed strong positive associations between LOD and age, there are substantial individual differences at each age. This study investigates whether nutrition and the possible risk factors for cataract previously mentioned could account for part of these individual differences. Fundus reflectance yields reliable estimates of the LOD.<sup>67,71</sup> It was used in this study, having the advantage that the MPOD was obtained simultaneously.

## METHODS

### SUBJECTS

The subjects in our study were recruited from the pool of volunteers of TNO Nutrition and Food Research and through advertising in local and regional newspapers and on television. The cross-sectional data analyzed herein were gathered during the baseline measurements of an intervention study designed to test an unrelated hypothesis. Advertising was aimed at recruiting people who have a general interest in participating in nutrition studies. Respondents who expressed potential interest received a questionnaire eliciting data on daily fruit and vegetable consumption and lifestyle factors. This questionnaire was designed for ranking subjects according to their intake and not for quantitative measurements. Only respondents in the highest quintile of fruit and vegetable consumption and those in the lowest tertile (calculated in the total group) were invited for an oral briefing and screening. In the lowest stratum, vitamin supplement users were excluded. The major inclusion criteria were age between 18 and 75 years and consumption of an average Western diet. The main exclusion criteria were pregnancy and/or lactation, wanting to become pregnant, serum cholesterol level greater than 290 mg/dL (>7.5 mmol/L) and/or triglyceride level greater than 204 mg/dL (>2.3 mmol/L) if not undergoing stabilized hypercholesterolemia or hyperlipidemia treatment, and anticoagulant therapy. A total of 380 volunteers were enrolled in the study. Two volunteers squinted too much during the reflectance measurement. Furthermore, one measurement failed for other reasons and one subject withdrew from the study before the reflectance measurement. Data from these 4 subjects were not included in the statistical analyses. Eight subjects were measured in the left eye instead of the right eye. Their measurements were included in the analysis, because there is a good correlation between both eyes.<sup>72</sup> The final study group comprised 177 men and 199 women. The study was performed according to the International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use guidelines for good clinical practice, and was approved by a medical ethical committee. Informed consent was obtained from all subjects.

### BLOOD AND ADIPOSE TISSUE SAMPLING

Blood samples from subjects were obtained between 8 AM and 9:30 AM, after an overnight fast. For the analysis of carotenoids and  $\alpha$ -tocopherol, blood was collected in tubes con-

taining clot activator and gel (Vacutainer Systems; Becton Dickinson, Franklin Lakes, NJ). These tubes were immediately stored in a closed box to avoid breakdown of carotenoids by UV light. Tubes were centrifuged within 15 to 30 minutes after collection at approximately 2000g for 10 minutes at approximately 4°C to obtain serum. After centrifugation, serum was removed and stored at approximately -80°C. Before storage, all serum was handled in subdued light.

For the analysis of vitamin C, blood was collected in tubes containing lithium heparin (Vacutainer Systems). A 0.5-mL aliquot of blood was added to 2 mL of metaphosphoric acid (50 g/L) (Mallinckrodt Baker, Deventer, the Netherlands) before freezing to preserve the vitamin C concentration during storage. This mixture was stored at approximately -80°C.

For the analysis of total cholesterol, blood was collected in tubes containing clot activator and gel (Vacutainer Systems). Tubes were centrifuged within 15 to 30 minutes after collection at approximately 2000g for 10 minutes at approximately 4°C to obtain serum. After centrifugation, serum was removed and stored at approximately -20°C.

Subcutaneous adipose tissue was obtained by needle biopsy from the lateral buttock,<sup>73</sup> using a 16-gauge needle attached to a plastic container in which the tissue was collected by connecting a vacuum tube. Samples were kept in the plastic container, immediately placed on dry ice, and stored at approximately -80°C.

### CHEMICAL ANALYSES

All carotenoids,  $\alpha$ -tocopherol, and vitamin C were analyzed by high-performance liquid chromatography. The coefficients of variation for the analysis of lutein, zeaxanthin, and  $\alpha$ -tocopherol were 3.7%, 11.5%, and 2.8%, respectively (at mean concentrations of 0.16, 0.03, and 20.90  $\mu$ mol/L in the quality control samples, respectively). The coefficient of variation for the analysis of vitamin C was 10%. Total cholesterol level was analyzed by enzymatic conversion to a stable chromogen, which could easily be detected by colorimetry with a commercially available kit (Boehringer Ingelheim, Mannheim, Germany). Broekmans et al<sup>74</sup> provide more details.

### MEASUREMENT OF LOD AND MPOD

Spectral fundus reflectance was measured with a densitometer (Utrecht Retinal Densitometer).<sup>75</sup> Briefly, a rotating wheel (14 revolutions per second) offered a sequence of 14 interference filters in the range of 430 to 740 nm to enable quasi-simultaneous measurement of the reflectance across the visual spectrum. Light reflected from the fundus was measured in a detection field of 1.5°, concentric within the illumination field of 1.8°. To obtain an estimate of the mean LOD and MPOD in this area, spectral fundus reflectance was measured under 2 conditions: (1) with the instrument's entry and exit pupils aligned to the peak of the Stiles-Crawford function and (2) 2 mm temporal to the Stiles-Crawford peak.<sup>71</sup> A detailed optical model of foveal reflection was used to arrive at individual estimates of variables such as the equivalent thickness of the blood layer, MPOD, LOD, and optical density of melanin.<sup>71</sup> In short, the incoming light was assumed to reflect at the inner limiting membrane, the discs in the outer segments of photoreceptors, and the sclera. By using known spectral characteristics of the different absorbers within the eye (lens, macular pigment, melanin, and blood), the optical densities of the pigments and the percentage reflectance at the interfaces were optimized to fit the measured data at all wavelengths. The spectral characteristic of the LOD was composed of a nonaging and an aging part.<sup>65</sup> Only the aging part was optimized in the fit procedure. The subjects' pupils were dilated with 0.5% tropicamide.

**Table 1. Characteristics and Lens Optical Density at 420 nm per Category**

Characteristic	No. of Subjects	Lens Optical Density, Mean ± SD	P Value
Sex			
Male	177	0.52 ± 0.17	.98
Female	199	0.52 ± 0.16	
Smoker			
No	264	0.52 ± 0.17	.66
Yes	112	0.52 ± 0.17	
Fruit and vegetable consumption*			
Low	214	0.51 ± 0.15	.16
High	162	0.54 ± 0.19	
Iris color			
Blue or gray	216	0.52 ± 0.17	.42
Blue, gray, or green	52	0.53 ± 0.18	
Green or light brown	25	0.57 ± 0.19	
Brown	26	0.55 ± 0.14	
Dark brown	12	0.46 ± 0.16	

\*Data obtained before the study.

#### MEASUREMENT OF OTHER CHARACTERISTICS

Smoking status was obtained by a questionnaire. Iris color was graded by a set of 4 standard photographs, providing a 5-grade classification system.<sup>76</sup> Weighing the subject wearing indoor clothing, without shoes, wallet, and keys, assessed body weight. Height was measured without shoes. The BMI was calculated as weight in kilograms divided by the square of height in meters.

#### STATISTICAL ANALYSES

The Statistical Product and Service Solutions software package, version 8.0.2 (SPSS Inc, Chicago, Ill), was used for data analysis. Pearson product moment correlation coefficients were calculated between continuous variables. To adjust for age effects, we also calculated partial correlation coefficients, controlling for age. *t* Tests and an analysis of variance were used to evaluate possible differences in continuous variables between men and women, between smokers and nonsmokers, between subjects with high and low fruit and vegetable intake, and between subjects with different iris colors.  $\chi^2$  Tests were used to evaluate possible differences between categorical baseline characteristics (sex, iris color, smoking status, and fruit and vegetable intake). If variables were not normally distributed, they were log transformed. Regression analysis was used to evaluate the association between categorical variables (sex, iris color, and smoking), covariates (age, MPOD, vitamins, and lutein, zeaxanthin, and cholesterol levels), and LOD.

#### RESULTS

The mean ± SD LOD at 420 nm was 0.52 ± 0.17. Study group characteristics are given in **Table 1** and **Table 2**. For ranking prestudy, the participants were classified into those with a diet low in fruit and vegetables, fruit juices included (57% of the study sample), and those with a diet rich in these foods (43% of the study sample). The latter group consisted of significantly more women (62%) than the former (46%) ( $P = .001$ ). The percentage of male smokers (32%) did not differ from that of female smokers (27%) ( $P = .33$ ). The LOD did not differ significantly between

men and women, between the groups with high and low fruit and vegetable intake, or between smokers and non-smokers (Table 1).

Iris color was classified in 331 participants. The participants predominantly (65%) had blue or gray irises. The distribution of subjects in the 5 iris categories did not differ between men and women ( $P = .72$ ). The LOD did not differ among the iris categories (Table 1).

Serum concentrations were significantly lower in men than in women for lutein, zeaxanthin, vitamin C, and total cholesterol. The lutein concentration in adipose tissue was also significantly lower in men than in women. The MPOD was significantly higher for men than for women (Table 2).

Age correlated most significantly with LOD ( $r = 0.71$ ,  $P < .001$ ), explaining 50% of the variance. Furthermore, serum and adipose lutein levels, serum  $\alpha$ -tocopherol level, height, BMI, and total cholesterol level also correlated significantly with LOD (Table 2). Other previously mentioned characteristics (MPOD and serum zeaxanthin and vitamin C levels) showed no association with LOD. We also found a significant correlation between age and serum lutein, serum  $\alpha$ -tocopherol, total cholesterol levels, BMI, and height (Table 2). Therefore, we calculated the partial correlation coefficient between all variables and LOD, controlling for age (Table 2). All previously mentioned correlations disappeared. Only MPOD showed a small significant negative correlation with LOD ( $r = -0.12$ ,  $P < .05$ ). This could imply that MPOD provided more detailed information on lutein and zeaxanthin content in the lens than on serum lutein, serum zeaxanthin, and adipose tissue lutein content. To test this hypothesis, we used linear regression analysis to identify factors that are associated most significantly with LOD. We used the forward conditional method with default selection criteria ( $P < .05$  to enter and  $P > .10$  to remove). Age, MPOD, and serum lutein, serum zeaxanthin, adipose tissue lutein, serum vitamin C, and  $\alpha$ -tocopherol levels were used as independent variables and LOD as the dependent variable. Total cholesterol level was included because of its correlation with serum carotenoids,  $\alpha$ -tocopherol, height, and BMI. Height, BMI, sex, smoking status, and iris color were also included. We only found significant  $\beta$  coefficients for age (mean ± SD,  $.008 \pm .0004$  [ $P < .001$ ]; 95% confidence interval [CI],  $.008-.009$ ) (16% per 10 years) and for MPOD (mean ± SD,  $-.096 \pm .041$  [ $P = .02$ ]; 95% CI,  $-.177$  to  $-.016$ ) ( $-2.7\%$  per SD in MPOD).

We stratified our data ( $\leq 50$  and  $> 50$  years) and applied the regression analysis on both strata. The mean ± SD LOD was  $0.68 \pm 0.16$  for the older subjects and  $0.45 \pm 0.11$  for the younger subjects. The mean ± SD MPOD was  $0.34 \pm 0.16$  and  $0.32 \pm 0.14$  for the older and younger subjects, respectively. For the older subjects ( $n = 114$ ), we found significant  $\beta$  coefficients for age (mean ± SD,  $.011 \pm .002$  [ $P < .001$ ]; 95% CI,  $.007-.015$ ) and MPOD (mean ± SD,  $-.240 \pm .086$  [ $P = .005$ ]; 95% CI,  $-.410$  to  $-.071$ ) ( $-5.6\%$  per SD in MPOD). For the younger subjects ( $n = 262$ ), we only found a significant  $\beta$  coefficient for age (mean ± SD,  $.006 \pm .0007$  [ $P < .001$ ]; 95% CI,  $.004-.008$ ), and there was no association between LOD and MPOD.

**Table 2. Descriptive Characteristics for the Total Study Group and Men and Women Separately\***

Characteristic†	Total (N = 376)	Men (n = 177)	Women (n = 199)‡	Correlation, r		
				With LOD	With Age	Partial§
Age, y	42 ± 14	42 ± 15	41 ± 13	0.71	...	...
MPOD (at 420 nm)	0.33 ± 0.15	0.35 ± 0.15	0.31 ± 0.14¶	-0.06	0.04	-0.12¶
Serum lutein level, µmol/L	0.18 ± 0.08	0.16 ± 0.07	0.19 ± 0.08#	0.19	0.26	0.003
Serum zeaxanthin level, µmol/L	0.049 ± 0.020	0.046 ± 0.018	0.052 ± 0.023#	0.06	0.05	0.04
Serum vitamin C level, mg/dL	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.03	-0.05	0.09
Serum α-tocopherol level, µmol/L	1.15 ± 0.26	1.12 ± 0.24	1.19 ± 0.28¶	0.30	0.45	-0.03
Adipose tissue lutein level, µmol/kg of wet weight**	0.40 ± 0.26	0.32 ± 0.17	0.47 ± 0.31	0.12¶	0.05	0.12
Height, m	1.75 ± 0.10	1.82 ± 0.08	1.69 ± 0.07	-0.18#	-0.21	-0.05
BMI	24.7 ± 4.3	24.5 ± 3.5	25.0 ± 4.9	0.14#	0.22	-0.02
Total cholesterol level, mg/dL	214 ± 37	208 ± 37	219 ± 36#	0.34	0.45	0.04

\*Data are given as mean ± SD unless otherwise indicated. LOD indicates lens optical density; MPOD, macular pigment optical density; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); and ellipses, data not applicable.

†To convert vitamin C to micromoles per liter, multiply by 56.78; to convert α-tocopherol to micromoles per liter, multiply by 23.22; and to convert total cholesterol to millimoles per liter, multiply by 0.02586.

‡Significance values refer to a comparison between men and women with the *t* test.

§Correlation with LOD, controlling for age.

||*P* = .001.

¶*P* = .05.

#*P* = .01.

\*\*Only determined in 187 subjects (89 men and 98 women).

## COMMENT

As expected, the LOD increased significantly with age. Furthermore, the MPOD showed an inverse relationship with LOD after adjustment for age. The inverse association between MPOD and LOD could reflect the greater likelihood of subjects with a high MPOD to have other healthy behaviors that retard lens aging. We found a significant, but small, correlation between MPOD and serum vitamin C level ( $r=0.12$ ,  $P=.02$ ). However, serum vitamin C level and other markers for a healthy lifestyle, which are possible risk factors for age-related cataract, like BMI and smoking, have been included in the regression analysis. Only MPOD showed a significant contribution.

Similar to Hammond et al,<sup>77</sup> we stratified our data ( $\leq 50$  and  $>50$  years). Only for the older subjects, we found a significant association between LOD and MPOD. To study this in more detail, we added an interaction term (age  $\times$  MPOD) in the regression analysis. However, the interaction term did not contribute significantly. It could be due to a nonlinear dependence of the LOD on age, not accounted for in the linear model analysis. Pokorny et al<sup>65</sup> determined, for subjects older than 60 years, a 3-fold increase in the rate at which the LOD changes as a function of age. In this study, we found the mean  $\pm$  SD  $\beta$  coefficient for age to be  $1.76 \pm 0.39$  times larger for subjects older than 50 years than for the younger subjects. For a cut point at age 60 years, we found the mean  $\pm$  SD  $\beta$  coefficient for age to be  $2.09 \pm 0.81$  times larger for the older subjects than for the younger subjects. We added an extra quadratic age term in the general linear model analysis, which indeed turned out to contribute significantly. However, the age  $\times$  MPOD interaction term remained nonsignificant. Thus, in the total study group, we could not find a significant change in the regression coefficient for the MPOD with age, although in the strati-

fied data the regression coefficient was only significant for the older subjects.

Like others,<sup>34,78</sup> we found a significantly higher MPOD in men than in women, despite lower serum and adipose tissue levels. Furthermore, like others,<sup>34,35</sup> we also found a higher correlation between MPOD and concentrations of serum and adipose tissue lutein in men than in women. Thus, the ability to transport lutein and zeaxanthin from the blood into the eye might be less in women than in men. It may be the reason that we found the LOD to show an inverse association with MPOD, rather than with serum and adipose tissue values of lutein and zeaxanthin. It may also be an explanation for the higher incidence of cataract in women than in men, apart from the impact of estrogen.<sup>79</sup>

We found no difference in LOD between smokers and nonsmokers. Hammond et al<sup>80</sup> studied LOD in relation to smoking behavior in younger subjects ( $n=41$ ) without cataract. They found a significant dose-response relationship between smoking frequency and LOD. Their data indicate that smoking is directly related to age-related increases in LOD throughout life, and that these increases persist even after smoking cessation. We had no information on duration of smoking and no information of ex-smokers. This may have lessened the strength of our analysis.

Hammond et al<sup>81</sup> also found significantly higher LODs in subjects with dark irises compared with subjects with light irises. We similarly divided our study group and found a mean  $\pm$  SD LOD of  $0.52 \pm 0.17$  for subjects with light irises ( $n=268$  [the first 2 categories in Table 1]) and of  $0.54 \pm 0.17$  for subjects with dark irises ( $n=63$  [the last 3 categories in Table 1]). Although the LOD was higher in subjects with dark irises, this difference was not significant ( $P=.42$ ). Hammond et al found only differences in LOD between light and dark irises for older subjects ( $>45$  years). Younger subjects showed no differ-

ences in LOD. We could not find any age effect in our study group. Model analysis of the reflectance spectra yielded a mean  $\pm$  SD retinal melanin optical density of  $0.87 \pm 0.18$  for subjects with light irises and of  $1.02 \pm 0.27$  for subjects with dark irises ( $P < .001$ ). The spectral absorbance of melanin and the aging component of the LOD show a slow decrease with increasing wavelength, without any characteristic spectral features. However, because of choroidal blood absorption, melanin shows its spectral fingerprint only for wavelengths greater than 600 nm, whereas the LOD is determined for wavelengths of less than 600 nm. As a consequence, their absolute values can be determined independently and reliably, and there is no indirect covariance between iris color and LOD from our variable fitting.

In summary, the LOD showed a significant association with age and MPOD. Macular pigment is composed of lutein and zeaxanthin, the only carotenoids found in human lenses. The MPOD is associated with serum and adipose lutein levels and can be modified by lutein intake. Inverse relationships between MPOD and LOD suggest that lutein and zeaxanthin in the eye may retard aging of the lens.

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## REFERENCES

- Apple DJ, Ram J, Foster A, Peng Q. Elimination of cataract blindness: a global perspective entering the new millennium. *Surv Ophthalmol*. 2000;45(suppl 1): S1-S196.
- Yeum KJ, Taylor A, Tang G, Russell RM. Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Invest Ophthalmol Vis Sci*. 1995;36: 2756-2761.
- Bates CJ, Chen SJ, Macdonald A, Holden R. Quantitation of vitamin E and a carotenoid pigment in cataractous human lenses, and the effect of a dietary supplement. *Int J Vitam Nutr Res*. 1996;66:316-321.
- Yeum KJ, Shang FM, Schalch WM, Russell RM, Taylor A. Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr Eye Res*. 1999; 19:502-505.
- Gerster H. Antioxidant vitamins in cataract prevention. *Z Ernährungswiss*. 1989; 28:56-75.
- Bunce GE, Kinoshita J, Horwitz J. Nutritional factors in cataract. *Annu Rev Nutr*. 1990;10:233-254.
- Jacques PF, Taylor A. Micronutrients and age-related cataracts. In: Bendich A, Butterworth CE, eds. *Micronutrients in Health and in Disease Prevention*. New York, NY: Marcel Dekker Inc; 1991:359-379.
- Tessier F, Moreaux V, Birlouez-Aragon I, Junes P, Mondon H. Decrease in vitamin C concentration in human lenses during cataract progression. *Int J Vitam Nutr Res*. 1998;68:309-315.
- Libondi T, Costagliola C, Della CM, et al. Cataract risk factors: blood level of antioxidative vitamins, reduced glutathione and malondialdehyde in cataractous patients. *Metab Pediatr Syst Ophthalmol*. 1991;14:31-36.
- Vitale S, West S, Hallfrisch J, et al. Plasma antioxidants and risk of cortical and nuclear cataract. *Epidemiology*. 1993;4:195-203.
- Gale CR, Hall NF, Phillips DIW, Martyn CN. Plasma antioxidant vitamins and carotenoids and age-related cataract. *Ophthalmology*. 2001;108:1992-1998.
- Lyle BJ, Mares-Perlman JA, Klein BE, et al. Serum carotenoids and tocopherols and incidence of age-related nuclear cataract. *Am J Clin Nutr*. 1999;69:272-277.
- Robertson JM, Donner AP, Trevithick JR. A possible role for vitamins C and E in cataract prevention. *Am J Clin Nutr*. 1991;53(suppl):346S-351S.
- Jacques PF, Chylack LT Jr. Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. *Am J Clin Nutr*. 1991;53(suppl): 352S-355S.
- Mares-Perlman JA, Brady WE, Klein BE, et al. Diet and nuclear lens opacities. *Am J Epidemiol*. 1995;141:322-334.
- Simon JA, Hudes ES. Serum ascorbic acid and other correlates of self-reported cataract among older Americans. *J Clin Epidemiol*. 1999;52:1207-1211.
- Hankinson SE, Stampfer MJ, Seddon JM, et al. Nutrient intake and cataract extraction in women: a prospective study. *BMJ*. 1992;305:335-339.
- Jacques PF, Taylor A, Hankinson SE, et al. Long-term vitamin C supplement use and prevalence of early age-related lens opacities. *Am J Clin Nutr*. 1997;66: 911-916.
- van der Pols JC. A possible role for vitamin C in age-related cataract. *Proc Nutr Soc*. 1999;58:295-301.
- Mares-Perlman JA, Lyle BJ, Klein R, et al. Vitamin supplement use and incident cataracts in a population-based study. *Arch Ophthalmol*. 2000;118:1556-1563.
- Chasan-Taber L, Willett WC, Seddon JM, et al. A prospective study of vitamin supplement intake and cataract extraction among US women. *Epidemiology*. 1999;10:679-684.
- Leske MC, Chylack LT Jr, Wu SY. The Lens Opacities Case-Control Study: risk factors for cataract. *Arch Ophthalmol*. 1991;109:244-251.
- Leske MC, Chylack LT Jr, He Q, et al. Antioxidant vitamins and nuclear opacities: the longitudinal study of cataract. *Ophthalmology*. 1998;105:831-836.
- Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report No. 9. *Arch Ophthalmol*. 2001;119:1439-1452.
- Tavani A, Negri E, La Vecchia C. Food and nutrient intake and risk of cataract. *Ann Epidemiol*. 1996;6:41-46.
- Brown L, Rimm EB, Seddon JM, et al. A prospective study of carotenoid intake and risk of cataract extraction in US men. *Am J Clin Nutr*. 1999;70:517-524.
- Chasan-Taber L, Willett WC, Seddon JM, et al. A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. *Am J Clin Nutr*. 1999;70:509-516.
- Lyle BJ, Mares-Perlman JA, Klein BE, Klein R, Greger JL. Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. *Am J Epidemiol*. 1999;149:801-809.
- Mares-Perlman JA, Brady WE, Klein BE, et al. Serum carotenoids and tocopherols and severity of nuclear and cortical opacities. *Invest Ophthalmol Vis Sci*. 1995;36:276-288.
- Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci*. 1988; 29:843-849.
- Handelman GJ, Dratz EA, Reay CC, van Kuijk JG. Carotenoids in the human macula and whole retina. *Invest Ophthalmol Vis Sci*. 1988;29:850-855.
- Berendschot TTJM, Goldbohm RA, Klöpping WA, et al. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci*. 2000;41:3322-3326.
- Bone RA, Landrum JT, Dixon Z, Chen Y, Llerena CM. Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Exp Eye Res*. 2000;71:239-245.
- Hammond BR Jr, Curran-Celentano J, Judd S, et al. Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res*. 1996;36:2001-2012.
- Johnson EJ, Hammond BR, Yeum KJ, et al. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr*. 2000;71:1555-1562.
- Hammond BR Jr, Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci*. 1997;38:1795-1801.
- Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp Eye Res*. 1997;65:57-62.
- West S, Munoz B, Emmett EA, Taylor HR. Cigarette smoking and risk of nuclear cataracts. *Arch Ophthalmol*. 1989;107:1166-1169.
- West S. Does smoke get in your eyes [editorial]? *JAMA*. 1992;268:1025-1026.
- Hankinson SE, Willett WC, Colditz GA, et al. A prospective study of cigarette smoking and risk of cataract surgery in women. *JAMA*. 1992;268:994-998.
- Klein BE, Klein R, Linton KL, Franke T. Cigarette smoking and lens opacities: the Beaver Dam Eye Study. *Am J Prev Med*. 1993;9:27-30.
- West S, Munoz B, Schein OD, et al. Cigarette smoking and risk for progression of nuclear opacities. *Arch Ophthalmol*. 1995;113:1377-1380.
- Christen WG, Glynn RJ, Ajani UA, et al. Smoking cessation and risk of age-related cataract in men. *JAMA*. 2000;284:713-716.
- Russell-Briefel R, Bates MW, Kuller LH. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am J Epidemiol*. 1985; 122:741-749.

45. Schechtman G, Byrd JC, Gruchow HW. The influence of smoking on vitamin C status in adults. *Am J Public Health*. 1989;79:158-162.
46. Seddon JM, Christen WG, Manson JE, et al. The use of vitamin supplements and the risk of cataract among US male physicians. *Am J Public Health*. 1994;84:788-792.
47. Caulfield LE, West SK, Barron Y, Cid-Ruzafa J. Anthropometric status and cataract: the Salisbury Eye Evaluation project. *Am J Clin Nutr*. 1999;69:237-242.
48. Schaumberg DA, Glynn RJ, Christen WG, Hankinson SE, Hennekens CH. Relations of body fat distribution and height with cataract in men. *Am J Clin Nutr*. 2000;72:1495-1502.
49. Mohan M, Sperduto RD, Angra SK, et al, and the India-US Case-Control Study Group. India-US case-control study of age-related cataracts [erratum appears in *Arch Ophthalmol*. 1989;107:1288]. *Arch Ophthalmol*. 1989;107:670-676.
50. Tavani A, Negri E, La Vecchia C. Selected diseases and risk of cataract in women: a case-control study from northern Italy. *Ann Epidemiol*. 1995;5:234-238.
51. Hankinson SE, Seddon JM, Colditz GA, et al. A prospective study of aspirin use and cataract extraction in women. *Arch Ophthalmol*. 1993;111:503-508.
52. Glynn RJ, Christen WG, Manson JE, Bernheimer J, Hennekens CH. Body mass index: an independent predictor of cataract. *Arch Ophthalmol*. 1995;113:1131-1137.
53. Hiller R, Podgor MJ, Sperduto RD, et al. A longitudinal study of body mass index and lens opacities: the Framingham Studies. *Ophthalmology*. 1998;105:1244-1250.
54. Klein BE, Klein R, Lee KE. Diabetes, cardiovascular disease, selected cardiovascular disease risk factors, and the 5-year incidence of age-related cataract and progression of lens opacities: the Beaver Dam Eye Study. *Am J Ophthalmol*. 1998;126:782-790.
55. Collman GW, Shore DL, Shy CM, Checkoway H, Luria AS. Sunlight and other risk factors for cataracts: an epidemiologic study. *Am J Public Health*. 1988;78:1459-1462.
56. Taylor HR, West SK, Rosenthal FS, et al. Effect of ultraviolet radiation on cataract formation. *N Engl J Med*. 1988;319:1429-1433.
57. The Italian-American Cataract Study Group. Risk factors for age-related cortical, nuclear, and posterior subcapsular cataracts. *Am J Epidemiol*. 1991;133:541-553.
58. Leske MC, Wu SY, Connell AM, Hyman L, Schachat AP. Lens opacities, demographic factors and nutritional supplements in the Barbados Eye Study. *Int J Epidemiol*. 1997;26:1314-1322.
59. Klein BE, Klein R, Lee KE. Incidence of age-related cataract: the Beaver Dam Eye Study. *Arch Ophthalmol*. 1998;116:219-225.
60. Chen CJ, Everett TK, Marascalco D. Snowflake degeneration: an independent entity or a variant of retinitis pigmentosa? *South Med J*. 1986;79:1216-1223.
61. Klein BE, Klein R. Cataracts and macular degeneration in older Americans. *Arch Ophthalmol*. 1982;100:571-573.
62. Sample PA, Esterson FD, Weinreb RN, Boynton RM. The aging lens: in vivo assessment of light absorption in 84 human eyes. *Invest Ophthalmol Vis Sci*. 1988;29:1306-1311.
63. Young RW. *Age-Related Cataract*. New York, NY: Oxford University Press; 1991.
64. Bron AJ, Vrensen GF, Koretz J, Maraini G, Harding JJ. The ageing lens. *Ophthalmologica*. 2000;214:86-104.
65. Pokorny J, Smith VC, Lutze M. Aging of the human lens. *Appl Opt*. 1987;26:1437-1440.
66. Johnson CA, Howard DL, Marshall D, Shu H. A noninvasive video-based method for measuring lens transmission properties of the human eye. *Optom Vis Sci*. 1993;70:944-955.
67. Delori FC, Burns SA. Fundus reflectance and the measurement of crystalline lens density. *J Opt Soc Am A Opt Image Sci Vis*. 1996;13:215-226.
68. van Norren D, Vos JJ. Spectral transmission of the human ocular media. *Vision Res*. 1974;14:1237-1244.
69. Cook CA, Koretz JF, Pfahnl A, Hyun J, Kaufman PL. Aging of the human crystalline lens and anterior segment. *Vision Res*. 1994;34:2945-2954.
70. Gaillard ER, Zheng L, Merriam JC, Dillon J. Age-related changes in the absorption characteristics of the primate lens. *Invest Ophthalmol Vis Sci*. 2000;41:1454-1459.
71. van de Kraats J, Berendschot TTJM, van Norren D. The pathways of light measured in fundus reflectometry. *Vision Res*. 1996;36:2229-2247.
72. Hammond BR Jr, Fuld K. Interocular differences in macular pigment density. *Invest Ophthalmol Vis Sci*. 1992;33:350-355.
73. Beynen AC, Katan MB. Rapid sampling and long-term storage of subcutaneous adipose-tissue biopsies for determination of fatty acid composition. *Am J Clin Nutr*. 1985;42:317-322.
74. Broekmans WMR, Berendschot TTJM, Klöpping WA, et al. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr*. 2002;76:595-603.
75. van Norren D, van de Kraats J. Retinal densitometer with the size of a fundus camera. *Vision Res*. 1989;29:369-374.
76. Seddon JM, Sahagian CR, Glynn RJ, Sperduto RD, Gragoudas ES, and the Eye Disorders Case-Control Study Group. Evaluation of an iris color classification system. *Invest Ophthalmol Vis Sci*. 1990;31:1592-1598.
77. Hammond BR Jr, Wooten BR, Snodderly DM. Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. *Optom Vis Sci*. 1997;74:499-504.
78. Hammond BR Jr, Caruso-Avery M. Macular pigment optical density in a Southwestern sample. *Invest Ophthalmol Vis Sci*. 2000;41:1492-1497.
79. Snow KK, Seddon JM. Age-related eye diseases: impact of hormone replacement therapy, and reproductive and other risk factors. *Int J Fertil Womens Med*. 2000;45:301-313.
80. Hammond BR Jr, Wooten BR, Nanez JE, Wenzel AJ. Smoking and lens optical density. *Ophthalmic Physiol Opt*. 1999;19:300-305.
81. Hammond BR Jr, Nanez JE, Fair C, Snodderly DM. Iris color and age-related changes in lens optical density. *Ophthalmic Physiol Opt*. 2000;20:381-386.