Lutein and Zeaxanthin Status and Risk of Age-Related Macular Degeneration

Catharine R. Gale, Nigel F. Hall, David I. W. Phillips, and Christopher N. Martyn

Purpose. To investigate the relation between plasma concentrations of lutein and zeaxanthin and age-related macular degeneration in a group of elderly men and women.

Methods. The Wisconsin Age-Related Maculopathy Grading System was used to grade features of early and late macular degeneration in 380 men and women, aged 66 to 75 years, from Sheffield, United Kingdom. Fasting blood samples were taken to assess plasma concentrations of lutein and zeaxanthin.

Results. Risk of age-related macular degeneration (early or late) was significantly higher in people with lower plasma concentrations of zeaxanthin. Compared with those whose plasma concentrations of zeaxanthin were in the highest third of the distribution, people whose plasma concentration was in the lowest third had an odds ratio for risk of age-related macular degeneration of 2.0 (95% confidence interval [CI] 1.0–4.1), after adjustment for age and other risk factors. Risk of age-related macular degeneration was increased in people with the lowest plasma concentrations of lutein plus zeaxanthin (odds ratio [OR] 1.9, 95% CI 0.9–3.5) and in those with the lowest concentrations of lutein (OR 1.7, 95% CI 0.9–3.3), but neither of these relations was statistically significant.

Conclusions. These findings provide support for the view that zeaxanthin may protect against age-related macular degeneration. (*Invest Ophthalmol Vis Sci.* 2003;44:2461–2465) DOI: 10.1167/iovs.02-0929

Age-related macular degeneration is the most common cause of blindness and visual impairment in elderly people in the developed world, but its etiology is poorly understood. In recent years, there has been increasing speculation that the xanthophyll carotenoids lutein and zeaxanthin may play a role in the pathogenesis of this disease. Lutein and zeaxanthin are the main constituents of the macular pigment of the retina.[1,2] Macular pigment is thought to protect against retinal damage by filtering out phototoxic short-wavelength visible light and by defending rod outer segment membranes from oxidative stress.[3–5] People with high macular pigment density have been shown to retain visual sensitivity at older ages,[6] and results of case–control studies suggest that such persons have a reduced risk of age-related macular degeneration.[7,8] There is evidence in humans that macular pigment density can be increased by raising the intake of lutein and zeaxanthin,[9–11] but whether these carotenoids can prevent the development of age-related macular degeneration remains unclear.

We used the Wisconsin Age-Related Maculopathy Grading System (WARMGS)[12] to assess macular changes in a group of elderly men and women. Our objective was to investigate the relation between plasma concentrations of lutein and zeaxanthin and the presence of age-related macular degeneration.

Methods

Participants

In recent years, the Medical Research Council (MRC) Environmental Epidemiology Unit has performed several studies on cohorts of people born in the Jessop Hospital for Women, Sheffield, United Kingdom, whose recorded birth measurements are still available. The members of these birth cohorts were traced in the National Health Service Central Register, and those still living in the city were invited to take part in research into the processes by which environment in early life influences adult disease.

We took the opportunity to examine the relation between plasma concentrations of lutein and zeaxanthin and age-related macular degeneration in a group of men and women aged 66 to 75 years who had taken part in one of these studies in Sheffield. One of the purposes of this study[13] had been to examine the relation between size at birth and risk of age-related macular degeneration. The study has been published. Briefly, we asked the Office for National Statistics to trace all 4793 people whose births were recorded between 1922 and 1930. Only those still living in Sheffield were eligible to take part in the study. A stratified sample of 746 people, comprising all 236 subjects from the highest and lowest fifths of birthweight and 85 randomly chosen subjects of each sex from each of the three intervening fifths of birthweight, was selected. After obtaining permission from their general practitioners, we wrote to 660 people asking whether we could interview them at home. Of those, 412 (62%) agreed to be interviewed by a field-worker. Of these, 392 (95%) were willing to attend a clinic.

Measurements

During the interview, the field-worker inquired about smoking habits, alcohol consumption, history of cardiovascular disease, education, and the most recent occupation of the participant or her husband. Height was measured with a portable stadiometer, and body weight was also measured.

At the clinic, fasting venous blood samples were taken for measurement of serum total cholesterol and plasma concentrations of carotenoids and vitamin E. All samples were stored at −80°C for later analysis.

Subsequently, plasma samples (500 mL) were treated with SDS (0.5 mL, 10 mM) and ethanol to precipitate plasma proteins. Carotenoids and vitamin E (as α-tocopherol) were extracted twice by the addition of hexane (2 mL), and the pooled hexane fraction was dried with a stream of nitrogen gas. The dry residue was dissolved in dichloromethane (100 μL) before adding 400 μL of acetonitrile-methanol (79:21 vol/vol). Pure commercial standards of carotenoids and α-tocopherol were used in the assays. Carotenoids and α-tocopherol were measured by high performance liquid chromatography[14] (HPLC; model 1100; Hewlett-Packard, Palo Alto, CA) The column system consisted of a

From the Medical Research Council Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton, United Kingdom.

Supported by the Wellcome Trust and the Medical Research Council.

Submitted for publication September 11, 2002; revised December 2, 2002, and January 17, 2003; accepted January 24, 2003.

Disclosure: **C.R. Gale**, None; **N.F. Hall**, None; **D.I.W. Phillips**, None; **C.N. Martyn**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Catharine R. Gale, Medical Research Council Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton, Hants SO16 6YD, UK; crg@mrc.soton.ac.uk.

10-mm, 5-μm metal-free guard column, with a 100 × 4.6-mm, 5-μm metal-free column (model ODS2; Hewlett-Packard) connected to a 250 × 4.6-mm, 5-μm analytical column (model 201TP54; Vydac, Hesperia, CA). The mobile phase consisted of acetonitrile, methanol, and dichloromethane (75:20:5 vol/vol/vol). Peak responses were measured at 450 nm (for carotenoids) and 294 nm (for α-tocopherol) using an ultraviolet-visible detector (model G1315A; Hewlett-Packard). Intra-assay variability was less than 8.5% for lutein, β-cryptoxanthin, β-carotene, and vitamin E, 15.4% for lycopene and 36.6% for zeaxanthin. Inter-assay variability was less than 15% for lutein, β-cryptoxanthin, β-carotene, and lycopene; 19% for vitamin E, and 46% for zeaxanthin. (The larger variability in the measurement of zeaxanthin was due to difficulties in separating its small peak and interference from minor components that occur in the same part of the chromatogram.)

Participants underwent an examination by an ophthalmologist who determined the refractive error by measuring the participants' usual distance glasses with a lens meter (model LM-350; Nidek Co., Ltd., Aichi, Japan) after first assessing each eye's visual acuity (at 4 m) with a logarithm of the minimum angle of resolution (logMAR) chart (Baillie-Lovie LogMAR chart; Lighthouse Enterprises, Long Island City, NY). Retinoscopy and subjective refraction were performed on all eyes that failed to read logMAR 1.2 or better. Participants who did not habitually wear distance glasses were assumed to be emmetropes if their unaided visual acuity was logMAR 1.2 or better. We calculated the spherical equivalent for each eye by adding the spherical error to half the cylindrical component.

The participants' pupils were dilated with tropicamide 1% and phenylephrine 2.5%, and two pairs of stereoscopic fundus photographs were taken of each eye on transparency film (E100S; Eastman Kodak, Rochester, NY) with a fundus camera (Carl Zeiss; Oberkochen, Germany) equipped with an Allen stereo-separator.[15] The stereo pairs were centered on the optic disc and on the center of the macula according to the recommendations of the WARMGS.[12] One observer (NH) graded each eye for the features of age-related maculopathy and age-related macular degeneration with the WARMGS, using a standard light box, stereo magnifier, and the grids and standard circles supplied by WARMGS. Features of early macular degeneration included the presence of large (>125-μm diameter) distinct, indistinct, confluent, or reticular drusen, or hypo- or hyperpigmentation. Features of late macular degeneration included either geographic atrophy or changes typical of exudative macular degeneration. The eyes were then categorized according to the presence of early or late macular degeneration, as defined by WARMGS.

Participants were defined as having early or late macular degeneration or no macular degeneration according to the status of their worse eye. Twelve participants were excluded from the analysis because of missing data ($n = 1$), myopic macular degeneration ($n = 4$), hypertensive maculopathy ($n = 3$), diabetic maculopathy ($n = 2$), or other non–age-related maculopathy ($n = 2$). The analyses that follow are therefore based on 380 participants.

The research adhered to the tenets of the Declaration of Helsinki. The study was approved by the South Sheffield Research Ethics Committee, and all participants gave written informed consent.

Statistical Analysis

We used the two-sample *t*-test (for continuous variables with a normal distribution), the Wilcoxon rank-sum test (for continuous variables with a skewed distribution), and the χ^2 test to analyze the relation between presence or absence of macular degeneration and potential risk factors. The potential risk factors examined in addition to plasma zeaxanthin, lutein, and lutein plus zeaxanthin were age, sex, body-mass index, pack years smoked, alcohol consumption (including beer and wine intake), serum total cholesterol, history of coronary artery surgery or angioplasty, social class, education, and average spherical equivalent. We used logistic regression to analyze the relation between plasma concentrations of lutein, zeaxanthin, and lutein plus zeaxanthin and risk of macular degeneration, with adjustment for other risk factors. In the multivariate models, we included all risk factors that were associated with the presence or absence of age-related macular degeneration with a significance level of $P < 0.10$. As lutein and zeaxanthin are lipid-phase nutrients and correlated with serum total cholesterol in these data ($r = 0.276$ and $r = 0.218$, respectively), serum total cholesterol was included in the multivariate analyses. Pack years smoked was also included in the multivariate analyses because it has been shown to be a risk factor for age-related macular degeneration in previous studies and was inversely related to plasma zeaxanthin levels in these data ($r = -0.109$). Odds ratios (OR; with 95% confidence interval [CI]) are shown according to thirds of the distribution of the lutein and zeaxanthin variables. Probabilities are shown for the trend in the odds ratios across the groups.

Results

Of the 380 participants (207 men and 173 women) included in the analysis, 78 (20.5%) had signs of either early or late age-related macular degeneration. Sixty-four (16.8%) participants had early and 14 (3.7%) had late macular degeneration. Because of the small number with late macular degeneration, early and late were combined for the analysis.

Table 1 shows the characteristics of the men and women who took part in the study, according to the presence or absence of macular degeneration. Compared with those without the disease, participants with macular degeneration were older and were more likely to have a history of coronary artery bypass grafting or angioplasty. Total alcohol consumption did not differ in people with or without macular degeneration, but a significantly larger proportion of those with the disease reported that they drank beer at least once a week. Participants with macular degeneration tended to be more hypermetropic than those without, but this difference was of borderline statistical significance. There were no statistically significant differences between the groups in sex, body-mass index, serum total cholesterol concentration, social class, education, or smoking habits, although men and women with macular degeneration tended to have smoked more cigarettes. Plasma concentrations of zeaxanthin were significantly lower in people with macular degeneration, 30 nM compared with 36 nM in those without the disease. Concentrations of lutein and lutein plus zeaxanthin were also lower, but these differences were not statistically significant.

We calculated odds ratios for age-related macular degeneration according to thirds of the distribution of plasma zeaxanthin, lutein, and lutein plus zeaxanthin. In the multivariate models we adjusted for all risk factors that were associated with macular degeneration with a $P < 0.10$ (age, beer consumption, history of angioplasty or coronary artery bypass grafting and hypermetropic refractive error). We also adjusted for serum total cholesterol, because zeaxanthin and lutein are lipid-phase nutrients, and for pack years smoked, because this has been shown to be a risk factor in previous studies and was inversely related to zeaxanthin levels in our participants ($r = -0.109$, $P = 0.034$).

Risk of age-related macular degeneration was highest in participants who had the lowest plasma concentrations of zeaxanthin (Table 2). Compared with those whose plasma zeaxanthin was in the highest third of the distribution (>46.3 nM), men and women whose plasma zeaxanthin was in the lowest third of the distribution (≤26.5 nM) had an odds ratio for age-related macular degeneration of 2.2 (95% CI 1.1–4.2), after adjustment for age. This relation remained statistically significant after further adjustment for other risk factors in multivariate analysis (*P* for trend = 0.046). The odds ratio for risk of age-related macular degeneration among those with the lowest zeaxanthin concentrations was 2.0 (95% CI 1.0–4.1),

TABLE 1. Characteristics of the Study Population, According to the Presence of Early or Late Age-Related Macular Degeneration

Characteristic	Absent (n = 302)	Present (n = 78)	P
Age (y)*	70.0 (2.1)	70.5 (2.4)	0.04
Male:female (% female)†	162:140 (46.4)	45:33 (42.3)	0.52
Body-mass index (kg/m^2)*	27.3 (4.6)	28.0 (4.5)	0.24
Cigarette smoking (pack-years)‡	15 (0–43)	19 (0–50)	0.26
Alcohol consumption (units per week)‡	7 (3–15)	6 (3–20)	0.62
Beer consumption (once a week or more) n (%)†	108 (35.8)	40 (51.3)	0.01
Wine consumption (once at week or more) n (%)†	65 (21.5)	12 (15.4)	0.20
Coronary artery surgery (CABG or angioplasty) n (%)†	13 (4.3)	9 (11.5)	0.02
Serum total cholesterol (mmol/L)	5.9 (1.3)	5.9 (1.3)	0.88
Plasma lutein (nmol/L)‡	177 (130–232)	153 (118–215)	0.16
Plasma zeaxanthin (nmol/L)‡	36 (24–55)	30 (20–46)	0.02
Plasma lutein plus zeaxanthin (nmol/L)‡	213 (160–287)	193 (147–256)	0.11
Social class (nonmanual) n (%)†	95 (31.5)	26 (33.3)	0.75
Education (stayed at school beyond age 14) n (%)	58 (19.3)	10 (12.8)	0.19
Average spherical equivalent (D)‡	1.0 (0–2.5)	1.8 (0–2.8)	0.07

CABG, coronary artery bypass graft.
* Mean (SD) with unpaired t-test for the equality of means.
† Number (%) with the characteristic of each group (χ^2 test).
‡ Median (interquartile range) with two-sample Wilcoxon rank-sum test statistic.

after adjustment for age and other risk factors. Plasma concentrations of lutein were not significantly associated with age-related macular degeneration, although the odds ratio was higher in participants in whom lutein levels were in the lowest third of the distribution (OR 1.7, 95% CI 0.9–3.3). There was no statistically significant relation between risk of age-related macular degeneration and levels of lutein plus zeaxanthin when we adjusted only for age. When the other risk factors were included in the multivariate model, the relation became slightly stronger but it was of borderline statistical significance (for trend, $P = 0.090$). Men and women whose plasma concentrations of lutein plus zeaxanthin were in the lowest third of the distribution (≤172.1 nM) had an odds ratio of 1.9 (95% CI 0.9–3.5), compared with those whose plasma concentrations were in the highest third of the distribution (>247.9 nM).

Participants with higher plasma concentrations of lutein or zeaxanthin also tended to have higher concentrations of vitamin E and the carotenoids β-carotene, lycopene, and β-cryptoxanthin. To investigate the possibility that one of these nutrients might explain the relations found between plasma zeaxanthin or plasma lutein plus zeaxanthin and risk of macular degeneration, we repeated our analyses adjusting for each of these potential confounders in turn and found little effect on estimates of risk (data not shown).

DISCUSSION

In this survey of men and women aged 66 to 75 years, we found a statistically significant trend between plasma concentrations of zeaxanthin and risk of age-related macular degeneration, after adjustment for age and other risk factors. People with the lowest plasma concentrations of this xanthophyll carotenoid had a two-fold increase in risk compared with those with the highest concentrations. There were no statistically significant trends between plasma concentrations of lutein alone or lutein plus zeaxanthin and age-related macular degeneration, though risk was nonsignificantly increased in people with the lowest concentrations.

Our study has some limitations. The cross-sectional design makes it impossible to be certain whether our assessment of

TABLE 2. Odds Ratios (95% CI) for Early or Late Age-Related Macular Degeneration, According to Plasma Concentrations of Lutein, Zeaxanthin, and Lutein plus Zeaxanthin

	n	Number with AMD (%)	Odds Ratio (95% CI), Adjusted for Age	Odds Ratio (95% CI), Multivariate Adjusted*
Zeaxanthin (nmol/L)				
≤26.5	124	33 (26.6)	2.2 (1.1–4.2)	2.0 (1.0–4.1)
26.6–46.3	123	27 (22.0)	1.7 (0.9–3.3)	1.7 (0.8–3.4)
>46.3	123	17 (13.8)	1.0	1.0
			P for trend = 0.019	P for trend = 0.046
Lutein (nmol/L)				
≤140.9	123	32 (26.0)	1.6 (0.9–2.9)	1.7 (0.9–3.2)
141.0–206.8	124	23 (18.5)	1.0 (0.5–1.9)	1.0 (0.5–1.9)
>206.8	123	22 (17.9)	1.0	1.0
			P for trend = 0.134	P for trend = 0.120
Lutein plus zeaxanthin (nmol/L)				
≤172.1	123	31 (25.2)	1.9 (1.0–3.2)	1.9 (0.9–3.5)
172.2–247.9	124	26 (21.0)	1.3 (0.7–2.6)	1.4 (0.7–2.7)
>247.9	123	20 (16.3)	1.0	1.0
			P for trend = 0.103	P for trend = 0.090

* Multivariate models include age, pack years smoked, serum cholesterol, beer consumption, history of angioplasty or coronary artery bypass grafting, and hypermetropic refractive error.

plasma lutein and zeaxanthin concentrations provides an accurate reflection of concentrations in the period before the development of macular degeneration. Although there was a reduced risk of age-related macular degeneration in people with higher plasma concentrations of zeaxanthin, it is possible that this was due, not to the protective effects of a high intake of zeaxanthin-rich foods but to some other characteristic of diet or way of life. Furthermore, our study was based on 392 participants who agreed to attend a hospital clinic—53% of the 746 people we selected to take part in the study. The men and women in our study cannot be considered representative of all elderly people in Sheffield, because they have continued to live in the city in which they were born. However, in the statistical analysis, all comparisons were made within the group who participated. We do not think that nonresponse or our inability to follow up all members of the original cohort biased the results, unless the relation between zeaxanthin status and age-related macular degeneration differs in nonresponders or in people who have died or moved away. Attrition of numbers, even if rates vary by disease status or risk factor, does not by itself bias estimates of risk. Bias arises only if attrition in a disease category varies according to risk factors. This variation might have happened in our study if, for example, people with both age-related macular degeneration and a high plasma concentration of zeaxanthin had decided that they would not take part. Our information about nonparticipants is limited to data contained in their birth records. However, the fact that there were no statistically significant differences in age between the 380 men and women included in our analyses (69.5 ± 2.1 years [SD]), the 20 individuals who agreed to be interviewed but did not attend our clinic (69.9 ± 2.0 years), and the 334 who were selected but did not participate in the study (69.8 ± 2.4 years) suggests that people with age-related maculopathy were not less likely to take part. Further reassurance on this point comes from the fact that the prevalence of early and late age-related macular degeneration in our study (16.8% for early macular degeneration and 3.7% for late macular degeneration) is similar to that found among people of the same age range in the Beaver Dam Eye study (18% and 1.4%, respectively).[16] Plasma concentrations of zeaxanthin and lutein plus zeaxanthin among our participants tended to be slightly lower than those recorded in the Beaver Dam study,[17] but this could be due to the higher proportion of smokers and elderly people in our study and to the fact that our measurements were made in fasting blood samples, rather than in nonfasting samples as were used in Beaver Dam.

Most previous studies that have examined the relation between lutein and zeaxanthin status and risk of age-related macular degeneration have analyzed the combined concentration of these two carotenoids, rather than looking at levels separately. Only one study found a significant association between blood concentrations of lutein plus zeaxanthin and risk of age-related macular degeneration. In the Eye Disease Case–Control Study of 421 cases and 615 control subjects, people with high serum concentrations of lutein plus zeaxanthin had a reduced risk of neovascular or exudative age-related macular degeneration.[18] In the third national health and nutrition examination survey (NHANES) of more than 8000 people, there was a nonsignificant inverse association between serum concentrations of lutein plus zeaxanthin and risk of late age-related macular degeneration, but this was not present for risk of two early signs of macular degeneration: soft drusen and pigmentary abnormalities.[19] Lutein and zeaxanthin concentrations were analyzed separately in a small case–control study nested in the Beaver Dam cohort. No differences were found between cases and controls, but these separate analyses were performed only on a subsample of participants, and, because of concern about lack of statistical power, it was decided not to proceed with separate analyses in the entire sample.[17] Our finding that risk of age-related macular degeneration was significantly higher in people with low levels of zeaxanthin, but not in those with low levels of lutein, suggests that the use of the sum of these xanthophyll carotenoids in previous investigations may have obscured evidence of zeaxanthin's protective role. It is possible that the results shown herein underestimate the true size of the risk associated with a poor zeaxanthin status. Imprecision in its measurement in our study would tend to bias estimates of risk toward unity.

Studies of the relation between diet and age-related macular degeneration have provided little evidence that lutein and zeaxanthin might be protective, but none of them have presented data for these carotenoids separately. High dietary intake of lutein and zeaxanthin combined was associated with a significantly lower risk of neovascular age-related macular degeneration in the Eye Disease Case–Control Study.[20] Risk was particularly low in those who were frequent eaters of spinach or collard greens, both rich sources of these carotenoids. In the third NHANES, significant inverse associations were found between consumption of lutein and zeaxanthin and risk of pigmentary abnormalities or late age-related macular degeneration, but only in the youngest age groups who were at risk of these conditions. There was no evidence in the study population as a whole that higher intakes of these carotenoids might protect against early or late age-related macular degeneration.[19] In the Beaver Dam Eye Study, there were no statistically significant correlations between intake of lutein and zeaxanthin and the prevalence of early or late age-related macular degeneration[21] or the incidence of early macular degeneration.[22]

Lutein and zeaxanthin may have different functions in the retina. Support for this comes from the fact that their distribution varies systematically, with zeaxanthin the dominant component in the central macula, whereas lutein dominates in the peripheral retina.[2,23] They also differ in their orientation within biological membranes.[24] Although both protect the lipid matrix from free radical attack, zeaxanthin has been shown to be a better photoprotector during prolonged exposure to ultraviolet radiation.[24] No data are available on lutein's ability to preserve photoreceptors in vivo, but in a recent study of quail, involving manipulation of the diet, higher retinal zeaxanthin markedly reduced light-induced photoreceptor cell death.[25]

Whether these xanthophyll carotenoids vary in the way in which they influence risk of age-related macular degeneration is not yet clear. Our finding that plasma zeaxanthin, but not plasma lutein, is significantly associated with risk of age-related macular degeneration suggests that their roles may not be identical.

Acknowledgments

The authors thank the participants for their time, Sheila Walton and Liz Kelleher for performing the fieldwork, Holly Syddall for statistical assistance, and the Institute of Food Research (Norwich, UK), for analysis of plasma carotenoids and vitamin E.

References

1. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. *Vision Res*. 1985;25:1531–1535.
2. Bone RA, Landrum JT, Friedes LM, et al. Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Exp Eye Res*. 1997; 64:211–218.
3. Kirschfeld K. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Proc R Soc Lond B*. 1982;216:71–85.

4. Rapp LM, Maple SS, Choi JH. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol Vis Sci*. 2000;41:1200–1209.
5. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys*. 2001;385:28–40.
6. Hammond BR, Wooten BR, Snodderly DM. Preservation of visual sensitivity of older subjects: association with macular pigment density. *Invest Ophthalmol Vis Sci*. 1998;39:397–406.
7. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci*. 2001;42:235–240.
8. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci*. 2001;42:439–446.
9. Hammond BR, Johnson EJ, Russel RM, et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci*. 1997; 38:1795–1801.
10. 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.
11. Berendschot TJM, Goldbohm AR, Klopping WA, Van de Kraats J, Van Norel J, Van Norren D. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci*. 2000;41:3322–3326.
12. Klein R, Davis MD, Magli YL, Segal P, Klein BEK, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991;98:1128–1134.
13. Hall NF, Gale CR, Syddall H, Martyn CN, Phillips DIW. Relation between size at birth and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2002;43:3641–3645.
14. Hart DJ, Scott KJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem*. 1995;54:101–111.
15. Karbassi M, Khu PM, Singer DM, Chylack LT. Evaluation of lens opacities classification system III applied at the slitlamp. *Optom Vis Sci*. 1993;70:923–928.
16. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology*. 1992;99:933–943.
17. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol*. 1995;113:1518–1523.
18. Eye Disease Case-Control Study Group. Antioxidant status and neovascular age-related macular degeneration. *Arch Ophthalmol*. 1993;111:104–109.
19. Mares-Perlman JA, Fisher AI, Klein R, et al. 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*. 2001;153:424–432.
20. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA*. 1994;272:1413–1420.
21. Mares-Perlman JA, Klein R, Klein BE, et al. Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch Ophthalmol*. 1996;114:991–997.
22. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BE, Brady WE, Palta M. 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*. 1998;148:204–214.
23. Bone RA, Landrum JT, Fernandez I, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci*. 1988;29:850–855.
24. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys*. 1999;371:301–307.
25. Thomson LR, Toyoda Y, Langner A, et al. Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Invest Ophthalmol Vis Sci*. 2002;43:3538–3549.