American Journal of Epidemiology

Vol. 153, No. 5
Printed in U.S.A.

Lutein and Zeaxanthin in the Diet and Serum and Their Relation to Age-related Maculopathy in the Third National Health and Nutrition Examination Survey

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Relations of the carotenoids lutein and zeaxanthin in the diet and serum to photographic evidence of early and late age-related maculopathy (ARM) among persons over age 40 years (n = 8,222) were examined. Inverse relations of these carotenoids in the diet or serum to any form of ARM were not observed overall. There was a direct relation of dietary levels to one type of early ARM (soft drusen). However, relations differed by age and race. In the youngest age groups who were at risk for developing early (ages 40–59 years) or late (ages 60–79 years) ARM, higher levels of lutein and zeaxanthin in the diet were related to lower odds for pigmentary abnormalities, one sign of early ARM (odds ratio among persons in high vs. low quintiles = 0.1, 95 percent confidence interval: 0.1, 0.3) and of late ARM (odds ratio = 0.1, 95 percent confidence interval: 0.0, 0.9) after adjustment for age, gender, alcohol use, hypertension, smoking, and body mass index. Relations of these carotenoids to ARM may be influenced by age and race and require further evaluation in separate populations and in prospective studies. *Am J Epidemiol* 2001;153:424–32.

carotenoids; diet; macular degeneration

Age-related macular degeneration (ARMD) is the most common cause of irreversible vision loss and legal blindness among older Americans (1). It is a degenerative condition of the region of the retina that is responsible for central vision (the macula). This condition, which has been estimated to affect about 0.5 percent of Americans over age 40 years (2), steeply increases in prevalence with age. Estimates from one United States community indicate that as many as 7 percent of persons between ages 75 and 84 years have ARMD (3). In this late stage, it usually cannot be successfully treated, and vision loss cannot be restored (4). Earlier stages of age-related maculopathy (ARM) are much more common, affecting about one quarter of people aged 65 years and older (3), and increase the risk of developing late ARM, also referred to as ARMD (5).

Received for publication October 18, 1999, and accepted for publication June 22, 2000.

Abbreviations: ARM, age-related maculopathy; ARMD, age-related macular degeneration; NHANES III, Third National Health and Nutrition Examination Survey; OR, odds ratio; RPE, retinal pigment epithelial.

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There is evidence to suggest that dietary intake of lutein and its structural isomer, zeaxanthin, could reduce the risk for ARM. These carotenoids are concentrated in the inner retinal layer of the macula (6) where the concentration is high and variable, ranging from approximately 0.1 to 1.0 pmole/square mm of tissue across individuals (7–9). The only source of these plant pigments, which impart a yellow color, is diet. Foods that contain them include leafy green vegetables, corn, green peppers, carrots, peaches, oranges, and egg yolks (10). Studies in humans and nonhuman primates suggest that the levels of lutein and zeaxanthin in the diet influence their levels in the macula (11–13).

In the retina, the two main mechanisms by which lutein and zeaxanthin might protect against ARM are by their ability to absorb light and to quench free radicals. These carotenoids, which are concentrated in the area of the retina where light exposure is most intense, may protect against light damage over many years by their ability to absorb and dissipate radiant energy. These pigments absorb light in the blue range (14, 15), which is thought to be particularly damaging to the retina (16). Damage from light exposure, particularly in the blue range where carotenoids absorb (17), has been related to ARM in some (17–19), but not all (20, 21), epidemiologic studies.

Another function of carotenoids that might explain their possible protective functions is their ability to quench free radicals or reactive oxygen species, thereby acting as an antioxidant (22). Reactive oxygen species are particularly high in the retina. This is due to sunlight, which promotes the development of free radicals, and to a high rate of oxidative metabolism, which produces free radicals as a by-product (23).

A protective role in preventing ARM was suggested by research indicating that levels of lutein and zeaxanthin in the diet and serum were lower in patients with one type of late stage ARMD in a previous multicenter study (24, 25). This is consistent with observations of lower levels of these carotenoids in autopsied eyes of people with compared with without macular degeneration (26). However, it is not clear whether the concentration of these pigments is related to earlier forms of ARM. To date, relations between levels of lutein and zeaxanthin in the diet and serum and earlier stages of ARM have not been demonstrated or sufficiently examined in previous epidemiologic studies (27–30).

We sought to determine the relations of lutein and zeaxanthin to both early and late ARM in the Third National Health and Nutrition Examination Survey (NHANES III). This was made possible by the availability of both retinal photographs and two separate markers for dietary carotenoid intake: estimates of intake from responses to a brief food frequency questionnaire and serum levels at the time of interview. We hypothesized that participants in the highest compared with the lowest quintiles of lutein and zeaxanthin in the diet would have a lower prevalence of both early and late ARM. We also hypothesized that similar relations would be observed with levels of these carotenoids in serum as well.

MATERIALS AND METHODS

Study population

NHANES III was conducted in two phases over a 6-year period (1988–1994) (31, 32). The sample is a stratified probability sample of the civilian noninstitutionalized United States population. Certain population subgroups, including Blacks, Mexican Americans, and adults aged 60 years and older, were oversampled so that stable estimates could be obtained for these groups individually. All potential survey participants were invited to participate in a home interview and a medical examination that took place in a mobile examination center. The home interview consisted of demographic, socioeconomic, and health history questions along with a 60-item food frequency questionnaire. The medical examination included, among other things, a physician's examination, fundus photography, blood and urine collection, and a 24-hour dietary recall interview.

A total of 14,464 persons aged 40 years and older were invited to participate in NHANES III. Of these, 11,448 (79 percent) were interviewed, 10,181 were examined, and 9,239 had one eye photographed. Included in the analyses reported in this paper are persons who had a gradable fundus photograph for ARM (8,603 persons), provided food-frequency questionnaires (8,596 persons), and provided blood for analyses of serum metabolites (8,229 persons, 57 percent of the original targeted sample).

To evaluate potential bias due to lack of participation in different aspects of NHANES III, we compared characteristics of participants who were included and those who were excluded from these analyses. Participants who were included were younger than those who were excluded (mean, 57 vs. 64 years). Therefore, all other comparisons were age adjusted. Significant differences ($p < 0.01$) in the characteristics of participants included compared with those excluded are as follows: Participants who were included were less likely to be current smokers (19 vs. 24 percent), but more likely to have smoked in the past (37 vs. 30 percent). A greater proportion of included participants were White (83 vs. 73 percent), and fewer were non-Hispanic Blacks (8 vs. 16 percent). Included participants were also less likely to have a history of hypertension (47 vs. 52 percent) or diabetes (9 vs. 13 percent). Nutritional characteristics also sometimes varied by inclusion status. Levels of vitamin E in the serum were higher in those included compared with those excluded (mean, 29 vs. 28 μmol/liter). The dietary intake of zinc (mean, 6.8 vs. 6.7 mg/day) and of lutein plus zeaxanthin was slightly higher (mean, 1,477 vs. 1,403 mg/day) in included subjects.

Fundus photography and grading

Protocols for obtaining and grading fundus photographs, described in detail elsewhere (2, 33), were adapted from the Wisconsin Age-Related Maculopathy Grading Scheme (34, 35). Briefly, the presence of any drusen, soft drusen, retinal pigment epithelial (RPE) depigmentation, increased retinal pigment, geographic atrophy, and signs of exudative macular degeneration (subretinal hemorrhage, subretinal fibrous scar, retinal pigment epithelium detachment, and/or serous detachment of the sensory retina) was determined. Two lesions associated with early ARM were quantified: soft drusen and pigmentary abnormalities (RPE depigmentation and increased retinal pigment). Soft drusen were defined by their diameter (larger than 63 μm). RPE depigmentation was graded as present or absent. Increased retinal pigment (the presence of granules or clumps of gray or black pigment in or beneath the retina) was graded as present or absent. Late ARM was defined as the presence of signs of exudative ARM degeneration or pure geographic atrophy (sharply delineated, roughly round or oval area of apparent absence of the RPE in which choroidal vessels are more visible than in surrounding areas).

Nutritional data

Intake of lutein plus zeaxanthin and other carotenoids was estimated from responses to food frequency questionnaires. This approach was taken in order to utilize estimates that represented a wide range of days because of the wide day-to-day variability that exists in intake of carotenoids. Nutrient composition databases utilized in creating these estimates were developed by using foods reported in the NHANES III 24-hour recalls. Foods reported in recalls were assigned to each food frequency question on the basis of the similarity of the food description and/or nutrient composition. Carotenoid levels were assigned based on the United States Department of Agriculture National Cancer Institute Carotenoid Food Composition Database (36). Some additional data values were obtained from a composite carotenoid database (37). Respondents were classified into 12 age- (ages 40–59, 60–79, and ≥80 years), sex-, and race-specific groups. (Those aged 80 years and older were

grouped by age and sex only because of the small number of participants in this age group.) Within each group, median nutrient values from 24-hour recalls were computed for each food item on the food frequency questionnaire.

Serum specimens collected during the mobile examinations and stored frozen were analyzed for levels of lutein and zeaxanthin (combined) and of other carotenoids and alpha-tocopherol by using reverse-phase, high-performance liquid chromatography with multiwavelength detection (38, 39). Serum carotenoids were log-transformed due to the skewness of their distribution. In addition, because lutein plus zeaxanthin levels were related to total serum cholesterol (Pearson correlation coefficient = 0.32), these values were regressed on serum cholesterol to compute residuals. These residual values were used in analyses to remove the variability in serum carotenoid that may have been attributed to differences in serum lipid levels. This adjustment had no substantive influence on the overall relations but sometimes narrowed the confidence intervals (data not shown).

Statistical analyses

Odds ratios and 95 percent confidence intervals for specific ARM endpoints (soft drusen, pigmentary abnormalities, and late ARM) were calculated for each quintile of carotenoid in diet and serum by using logistic regression, with those in the lowest quintile as the reference category. For late ARM, analyses were restricted to people aged 60 years and older because there was only one person younger than age 60 who had late ARM. Quintiles of carotenoid in the diet were assigned by age-decade to remove the influence of differences in intake due to decreasing energy needs across decades. Linear trends were assessed by using quintile medians. Age (entered continuously) was included in all regression models. We also evaluated the influence of potential risk factors that differed among people within the high and the low quintiles for carotenoids in the diet or serum (table 1). These included gender, smoking (current, past, never), eye color (blue vs. nonblue), alcohol intake (drinks/month), history of heavy drinking (yes/no), hypertension (blood pressure greater than 140/90 mmHg and/or use of hypertension medications; yes or no), diabetes (self-reported presence or absence), body mass index (measured weight (kg)/height $(m)^2$; continuous), cardiovascular disease (self-reported presence or absence), and use of estrogens or progesterone (women only; ever vs. never). After adjustment for age, any of these factors (entered singly) that influenced odds ratios by 10 percent or more for any lesion were retained in the final model. Only gender influenced odds ratios to this degree. We also included in the final adjusted model any additional factors that were markedly different across levels of lutein and zeaxanthin in diet and serum.

We tested for interactions to determine whether the associations between carotenoids and ARM differed according to the levels of other risk factors. An alpha level of 0.10 was used to determine significance. Because significant age and race interactions were apparent (as is discussed in Results), analyses were conducted both in the overall group and in specific age- and race-specific subgroups.

Because of concern for type II errors, significance levels were not modified to take into account multiple testing. Total interview or examination sample final weights (32) were applied to all estimators to account for individual selection probabilities, nonresponse, and poststratification. In addition, to take into account clustering that resulted from the complex survey design in the NHANES III, we used the jackknife replication method to obtain appropriate variance estimates in regression analyses (32).

RESULTS

Distribution of lutein and zeaxanthin

The distributions of lutein plus zeaxanthin in serum and diet, by race, are shown in table 2. The distributions of intakes were markedly higher among non-Hispanic Blacks. Levels in the serum were generally lowest among non-Hispanic Whites and highest among non-Hispanic Blacks. However, the magnitude of differences in the distributions across races was less marked than the differences in the distributions in the diet.

Distribution of risk factors by level of lutein and zeaxanthin

We investigated the presence of other previously identified potential risk factors for ARM among people in high versus low quintiles for levels of lutein and zeaxanthin in the serum and diet (table 1). Relations of potential risk factors to these carotenoids in the diet and serum generally followed similar patterns; having lutein and zeaxanthin in the diet or serum in high quintiles compared with low quintiles was associated with a lower prevalence of potential risk factors for ARM, including current smoking, female gender, moderate overweight, obesity (serum only), and history of hypertension (serum only). Higher lutein and zeaxanthin levels were associated with potential nutritional protective factors for ARM, including higher levels of beta-carotene (diet and serum), higher alpha-tocopherol (serum), higher zinc (diet), and lower fat (as percent of energy in diet). Consequently, we evaluated the influence of adjusting relations of lutein and zeaxanthin to ARM for these nondietary and dietary risk or protective factors in subsequent analyses (described below.)

Relations to early ARM lesions

The adjusted odds ratios for two early signs of ARM (soft drusen and pigmentary abnormalities) by quintile of lutein and zeaxanthin intake in the total sample are given in the first row of table 3. These adjusted odds ratios were almost identical to odds ratios after simple adjustment for age (data not shown). Pigmentary abnormalities were not associated with levels of lutein plus zeaxanthin in the diet or serum. Soft drusen was directly related to levels of these carotenoids in the diet (adjusted odds ratio = 1.4, 95 percent confidence interval: 1.0, 1.8) among those in high versus low quintiles in the overall sample. However, serum levels of these carotenoids were generally unrelated to the pres-

TABLE 1. Weighted and age-standardized adjusted rates and means by level of lutein and zeaxanthin in serum and diet in NHANES III† participants, 1988–1994

	Lutein and zeaxanthin levels			
	Serum quintile		Diet quintile	
	1	5	1	5
Smoking (%)				
Past	30	38*	33	38*
Current	30	12*	24	20*
Body mass index‡ (%)				
Overweight	26	18*	25	23
Obese	23	11*	18	15
History of cardiovascular disease (%)	16	12	14	15
History of hypertension (%)	52	42*	48	47
History of diabetes (%)	11	8	9	11
Gender, (female) (%)	60	56‡	55	51*
Serum§				
LDL cholesterol† (mmol/liter) (mean)	3.60	3.47*	3.57	3.46
Beta-carotene (μmol/liter) (mean)	0.22	0.52*	0.27	0.40*
Vitamin E (μmol/liter) (mean)	26	31*	28	29*
Diet§				
Beta-carotene (μg/day) (mean)	1,774	2,962*	1,060	4,934*
Fat (% energy) (mean)	34	32*	34	32*
Zinc (mg/day) (mean)	6.39	6.91*	5.80	7.89*
Energy (kcal/day) (mean)	1,628	1,766*	1,705	1,756
Menopausal women aged 40–49 years (%)				
Premenopausal	21	22	21	21
Postmenopausal	5	3	3	4
Postmenopausal women using estrogen at time of examination (%)	13	19	15	15

* $p < 0.05$ for difference across quintiles.

† NHANES III, Third National Health and Nutrition Examination Survey; LDL cholesterol, low density lipoprotein cholesterol.

‡ Overweight: body mass index of 27.8–31.0 for males and 27.3–32.2 for females: obese: body mass index >31.0 for males and >32.2 for females.

§ Geometric mean values are given.

ence of soft drusen. Therefore, we rejected our a priori hypothesis that lutein and zeaxanthin in the diet and in serum would be related to lower rates of early forms of ARM in the overall NHANES III sample.

To explore the influence of recent dietary changes that may be more common among people with comorbid conditions (cardiovascular disease, hypertension, or diabetes mellitus), we repeated analyses after excluding people who indicated a history of any of these conditions. This reduced the sample from 8,012 to 3,551 people. Odds ratios for pigmentary abnormalities were further from the null but remained statistically nonsignificant (odds ratio (OR) = 0.7, 95 percent confidence interval: 0.4, 1.3 before and OR = 0.4, 95 percent confidence interval: 0.07, 1.2 after exclusions and adjustment for factors described in table 3). Odd ratios for soft drusen were similar (OR = 1.4, 95 percent confidence interval: 1.1, 1.8 before and OR = 1.3, 95 percent confidence interval: 0.9, 2.1 after these exclusions). Limiting analyses to people who reported dietary changes associated with chronic disease in the year before interviews also did not influence associations (data not shown).

Odds ratios differed considerably across age and race groups, and significant interaction effects were noted for age and race (table 3). Therefore, we evaluated relations in separate age and race strata, with particular focus on the younger ages. In younger persons, relations might be less likely to reflect a knowledge of retinal changes and recent diet change due to these or other health conditions. Relations in this group would also be less likely to reflect a selective mortality bias, if one exists. In the youngest age group (ages 40–59 years), persons in high quintiles for dietary lutein and zeaxanthin had a 90 percent lower risk for pigmentary abnormalities compared with those in the lowest quintile (age- or fully adjusted odds ratio = 0.1, 95 percent confidence interval: 0.1, 0.3). Relations with serum lutein and zeaxanthin were in a similar direction, but were not statistically significant (adjusted odds ratio = 0.5, 95 percent confidence interval: 0.1, 1.7). In the older age groups, the

TABLE 2. Unweighted distribution* of lutein and zeaxanthin in NHANES III† participants aged 40 years and older by age and race, 1988–1994

	Serum‡ (μmol/liter)			Diet (μg/day)		
	10th Percentile	50th Percentile	90th Percentile	10th Percentile	50th Percentile	90th Percentile
Ages 40–59 years	0.21	0.38	0.67	394	1,592	5,554
Non-Hispanic White	0.19	0.34	0.61	315	1,291	4,287
Non-Hispanic Black	0.21	0.39	0.64	699	2,815	8,532
Mexican American	0.23	0.40	0.69	459	1,136	3,400
Ages 60–79 years	0.22	0.40	0.72	441	1,640	5,973
Non-Hispanic White	0.19	0.35	0.64	389	1,355	4,905
Non-Hispanic Black	0.24	0.43	0.79	872	3,425	9,715
Mexican American	0.22	0.39	0.68	452	1,240	3,445
Ages ≥80 years	0.22	0.40	0.77	382	1,443	5,601
Non-Hispanic White	0.20	0.36	0.69	382	1,364	4,730

* These unweighted values are used to better reflect the impact of these levels on analysis but do not represent national estimates of the distribution of these carotenoids.

† NHANES III, Third National Health and Nutrition Examination Survey.

‡ Serum lutein was first log-transformed to approach normality and then regressed on total serum cholesterol. Residuals from this regression were then added to race-specific medians and back-transformed to the original scale to achieve these distributions.

relations of these carotenoids in the diet or serum to pigmentary abnormalities were closer to the null.

When analyses were conducted in separate race strata with the age group 40–59 years, an inverse direction for the relation of diet lutein and zeaxanthin to pigmentary abnormalities was apparent only among non-Hispanic Whites and Mexican Americans (table 3). However, confidence intervals were wide, particularly among Mexican Americans and non-Hispanic Blacks, among whom there were few cases. There were fewer than 20 non-Hispanic Blacks and Mexican Americans in this age group who had these lesions.

To investigate whether higher intake of lutein and zeaxanthin in Blacks and Mexican Americans compared with non-Hispanic Whites explained the lower prevalence of pigmentary abnormalities in these groups (4) compared with non-Hispanic Whites, we computed odds ratios before and after adjusting for these dietary carotenoids. The odds ratio for pigmentary abnormalities in Mexican Americans and non-Hispanic Blacks compared with non-Hispanic Whites (OR = 0.7, 95 percent confidence interval 0.4, 1.1 and OR = 0.7, 95 percent confidence interval: 0.5, 1.1, respectively) were identical before and after adjustment.

Relations of lutein and zeaxanthin to soft drusen were also influenced by age (table 4). The direct relations that were observed in the overall group were generally strongest among those aged 80 years or more, the majority (81 percent) of whom were non-Hispanic Whites. Among the youngest participants of each race, relations of these dietary carotenoids to soft drusen were either nonexistent or weak.

Relations to late ARM

Nonsignificant inverse associations were observed between levels of lutein and zeaxanthin in the diet or serum and late ARM (table 4, first row). We stratified by age in order to explore the possibility that selective mortality bias might influence these associations. We observed lower odds for late ARM among people aged 60–79 years with these diet carotenoids in the high versus the low quintiles. The odds ratios were 0.1 (95 percent confidence interval: 0.0, 0.1) after adjustment for age and remained unchanged (OR = 0.1, 95 percent confidence interval: 0.0, 0.9) after adjustment for other risk factors. Odds ratios in older persons (aged ≥80 years) were inverse but were not statistically significant. Relations of lutein and zeaxanthin in the serum to late ARM followed patterns similar to those with dietary levels, but were closer to the null.

Influence of other diet attributes

Next, we evaluated the possibility that the inverse relations of lutein and zeaxanthin in the diet to pigmentary abnormalities and late ARM in the younger age groups might reflect relations of other dietary factors to ARM. To achieve convergence of these models, only age and the level of nutrient in the diet (beta-carotene or zinc estimates from food frequency questionnaires or dietary saturated fat from 24-hour recalls) or serum (vitamin E) were added as covariates in these analyses. When the regression models did not converge for late ARM (for dietary zinc and saturated fat), the presence of levels of dietary intakes that were at or above versus below the median for these nutrients were substituted for the continuous value of these variables. Adjustment for each potential dietary confounder singly had no influence on odds ratios (data not shown).

DISCUSSION

Dietary lutein and zeaxanthin

We did not observe hypothesized relations between lutein and zeaxanthin in the diet and either type of early ARM or

TABLE 3. Adjusted* odds ratios and 95% confidence intervals for macular soft drusen and pigmentary abnormalities among NHANES III† participants in high versus low quintiles of lutein and zeaxanthin in the serum and diet, 1988–1994

	Soft drusen								Pigmentary abnormalities							
	Serum				Diet				Serum				Diet			
	No. at risk	No. with outcome	OR†	95% CI†	No. at risk	No. with outcome	OR	95% CI	No. at risk	No. with outcome	OR	95% CI	No. at risk	No. with outcome	OR	95% CI
All races combined‡																
All ages	7,559	817	1.0	0.6, 1.5	7,897	868	1.4	1.0, 1.8§	7,669	210	0.9	0.5, 1.5¶	8,012	218	0.7	0.4, 1.3¶
40–59 years	3,662	190	0.7	0.3, 1.7	3,816	197	1.2	0.6, 2.3	3,675	49	0.5	0.1, 1.7¶	3,829	51	0.1	0.1, 0.3
60–79 years	3,139	430	1.1	0.6, 1.9	3,278	461	1.3	0.9, 1.9	3,202	87	1.4	0.6, 3.4	3,344	90	1.1	0.5, 2.4
≥80 years	758	197	1.4	0.7, 2.7	803	210	2.4	1.3, 4.4	792	74	0.9	0.4, 2.0	839	77	1.0	0.4, 2.4
Non-Hispanic Whites																
All ages	3,883	489	1.2	0.9, 1.7	4,006	510	1.3	1.0, 1.8	3,934	151	0.8	0.4, 1.3	4,059	155	0.9	0.5, 1.5
40–59 years	1,568	64	0.9	0.3, 2.3§	1,608	64	0.9	0.4, 2.0	1,570	21	0.7	0.2, 3.0	1,610	22	0.3	0.1, 0.3
60–79 years	1,692	248	1.3	0.8, 2.0	1,745	260	1.2	0.8, 1.7	1,716	61	0.7	0.3, 1.7	1,769	62	0.9	0.4, 2.0
≥80 years	623	177	1.4	0.8, 2.4	653	186	2.1	1.2, 3.7	648	69	0.8	0.4, 1.7	680	71	1.3	0.6, 2.8
Non-Hispanic Blacks																
All ages	1,738	137	1.0	0.5, 1.7	1,882	158	1.4	0.8, 2.3	1,765	26	2.3	0.6, 9.0	1,912	28	2.8	0.9, 9.1
40–59 years	1,021	56	1.5	0.7, 3.5	1,098	63	1.0	0.5, 2.3	1,028	12	2.7	0.3, 2.7	1,105	13	1.2	0.2, 6.4
60–79 years	655	69	0.6	0.3, 1.5	712	80	1.2	0.6, 2.4	671	11		#	731	12		#
Mexican Americans																
All ages	1,658	155	1.1	0.6, 1.9	1,715	163	1.3	0.7, 2.2	1,688	27	2.6	0.8, 7.7	1,745	29	0.8	0.2, 2.7
40–59 years	910	50	1.3	0.5, 3.6	939	50	1.4	0.5, 3.6	914	13		#	943	13	0.5	0.0, 5.1
60–79 years	693	98	1.1	0.5, 2.1	718	105	1.1	0.5, 2.2	715	13	4.8	0.5, 47	740	14		#

* Adjusted for age, gender, alcohol use, hypertension, smoking, and body mass index.
† NHANES III, Third National Health and Nutrition Examination Survey; OR, odds ratio; CI, confidence interval.
‡ Analyses applied sample weights and jackknife method of variance estimation to account for complex design.
§ Odds ratios were different across age groups ($p < 0.10$).
¶ Odds ratios were different across race groups ($p < 0.10$).
# Logistic regression models did not converge in this subgroup, which contained few persons with this outcome.

TABLE 4. Adjusted* odds ratios and 95% confidence intervals for late age-related maculopathy among NHANES III† participants in high versus low quintiles for lutein and zeaxanthin in the serum and diet, 1988–1994

All races combined‡	Serum				Diet			
	No. at risk	No. with late ARM†	OR†	95% CI†	No. at risk	No. with late ARM	OR	95% CI
Age (years)								
≥60	3,685	45	0.6	0.2, 1.9	3,857	47	0.5	0.1, 1.6
60–79	3,012	15	0.5	0.1, 5.1	3,143	16	0.1	0.0, 0.9
≥80	673	30	0.8	0.3, 2.4	714	31	0.6	0.2, 2.7

* Adjusted for age, gender, alcohol use, hypertension, smoking, and body mass index.

† NHANES III, Third National Health and Nutrition Examination Survey; ARM, age-related maculopathy; OR, odds ratio; CI, confidence interval.

‡ Analyses applied sample weights and jackknife method of variance estimation to account for complex design. There were too few non-white participants with late ARM (9 people) to permit stratification by race.

late ARM in the overall NHANES III sample. However, inverse relations of intake of these carotenoids to some ARM lesions (pigmentary abnormalities and late ARM) were observed when the analyses were limited to the youngest age groups who were at risk for these conditions (ages 40–59 and 60–79 years, respectively). The possibility that these dietary carotenoids protect against later stages of ARM is consistent with results from one case-control study in a previous population in which lutein and zeaxanthin levels in both the diet (24) and serum (25) were related to lower odds for one type of late ARM (neovascular/exudative macular degeneration), but not to a smaller nested case-control study (28). Protective roles for carotenoid pigments in the macula are also suggested by lower levels of these carotenoids in autopsied eyes from people who had a history of ARMD than in those without (26). If the inverse relations that were limited to younger participants are real, then our study extends these findings to one form of earlier ARM, pigmentary abnormalities. Pigmentary abnormalities, like large soft drusen, have been observed to predict incident late ARM over 5 years (5). The existence of pigmentary abnormalities is less common and is thought to represent a later stage in the natural history of ARM than is soft drusen. Some have speculated that pigmentary abnormalities indicate the poor health of the RPE cells prior to their death.

Inverse relations between lutein and zeaxanthin in the diet or serum and early signs of ARM have not been observed previously in separate studies of a Wisconsin community (27, 28). This may be due to the narrower range of lutein and zeaxanthin intake in this Wisconsin sample relative to the American population. The range in serum levels between the 10th and 90th percentiles in the Wisconsin sample was 0.30 μmol/liter compared with 0.53 μmol/liter in the NHANES III sample.

This limitation of the relations between dietary lutein and zeaxanthin and pigmentary abnormalities to the youngest subjects could reflect chance, selective mortality, selective cohort bias, or a greater likelihood of recent diet changes (such as increases in fruit and vegetable intakes) among older participants, who are more likely to have early retinal changes or comorbid conditions. Exclusion of individuals with a history of cardiovascular disease, diabetes, or cancer or with reported dietary changes due to chronic disease in the previous year had little effect on our findings but also reduced statistical power. We could not completely identify people who have made recent diet changes. This may limit our ability to identify inverse associations in these cross-sectional analyses.

Inverse relations of lutein and zeaxanthin intake to ARM were not observed in Black Americans, in whom pigmentary abnormalities and late ARM were less common than in Whites in the NHANES III sample (4) and in other investigations (40, 41). Lower susceptibility in Black Americans may be because of higher earlier death rates due to cardiovascular disease (42), which has sometimes been related to risk for ARM (20, 21, 43). Alternatively, more highly pigmented persons may have developed protective mechanisms in the eye to handle chronic sun exposure, such as higher ocular melanin. Lower ocular melanin has been suggested as an explanation for high prevalence of late-stage macular degeneration in Whites (44).

We considered the possibility that the lower occurrence of pigmentary abnormalities in Black and Mexican American, compared with White, populations may actually be related to differences in the intake of carotenoids. The proportion of people with intakes and blood levels in the low ranges was highest among non-Hispanic White Americans (table 3). However, racial differences in the odds of pigmentary abnormalities were not influenced by adjustment for these dietary carotenoids.

The observation in our analyses of a direct relation of lutein and zeaxanthin intake to the earliest and most common form of ARM, soft drusen, weakens the possibility that dietary lutein and zeaxanthin protect against ARM. However, it is possible that this relation reflects recent increases in fruit and vegetable intakes as a consequence of knowing about these retinal abnormalities or having comorbid conditions. This notion is supported by the fact that direct relations are strongest in the oldest age groups. Moreover, intake of these carotenoids was not related to prevalent (27) or incident (29) drusen in the Beaver Dam Eye Study. In a separate case-control study of ARM, which included mainly cases with

drusen, a nonsignificant inverse relation between serum lutein and zeaxanthin and ARM was observed (30). Therefore, the current body of evidence is not consistent with a direct relation of lutein and zeaxanthin intake to the presence of macular drusen.

Serum lutein and zeaxanthin carotenoids

Similar, but weaker, associations between lutein and zeaxanthin in the serum and ARM were observed in our analyses. One possible reason for weaker associations with carotenoids in the serum compared with diet could be that these carotenoids are not causally related to ARM, but are markers for other dietary patterns. In other populations, dietary attributes that are related to higher lutein and zeaxanthin intake (higher intakes of provitamin A carotenoids (24, 25, 42), zinc (27, 29), and vitamin E (29, 45) and lower intakes of saturated fat (46)) have been related to lower rates of some forms of ARM. However, adjustments for the intake of these nutrients did not substantively weaken the inverse associations of these dietary carotenoids to pigmentary abnormalities and late ARM. Nonetheless, inevitable error in the measurement of these dietary factors may have reduced our ability to sufficiently account for these other aspects of diet in the regression model. That dietary lutein and zeaxanthin correlate with a general dietary pattern that helps to protect against ARM in one or more ways remains a possibility.

A second explanation for the observation of weaker associations with levels of carotenoids in the serum compared with diet could be that these measures reflect different periods of time. Levels in the serum reflect intake over a week or two, whereas food frequency questionnaire responses were intended to reflect intake over a month and probably reflect an even longer period of time.

A third explanation for weaker associations with carotenoids in the serum is that these values reflect a variety of physiologic factors that are unrelated to the levels that are available to the retina. Evidence for this possibility is that levels of lutein were lower in the body fat and higher in the retinas of men compared with women, despite similar levels of lutein in the serum in one previous study (47). The factors that influence the uptake and distribution of carotenoids are still poorly understood, so it is not yet possible to adjust for all such factors that influence levels of these carotenoids in the blood in epidemiologic analyses.

Limitations

There were several limitations of this investigation that may influence conclusions drawn from the results. The analyses included only 57 percent of the original targeted sample because of nonparticipation in the overall survey or in various parts of the survey integral to these analyses. Therefore, nonresponse bias is possible. Without estimates of ARM in the unsurveyed population, it is not possible to fully evaluate or completely adjust for this possible bias. When comparing NHANES III participants who were included and those who were excluded from these analyses, we observed that those included were younger and had higher intakes of lutein and zeaxanthin. This suggests that the relations of carotenoids in the serum to ARM may be biased toward the null.

Second, these analyses were cross-sectional, and this design may be particularly problematic when analyzing relations of conditions that develop over the long term and are strongly related to increasing age, as is macular degeneration. A declining proportion of individuals who are susceptible to ARM in the general population at older ages could bias associations observed. Differences in associations across ages suggests that this bias may exist. This limited the size of the sample in which associations could be validly evaluated, leading to a higher likelihood of both type I and type II errors that could have influenced results in these smaller subgroups. A third limitation is the large number of analyses performed in subgroups, so that some associations observed could be the result of chance.

Summary

In summary, we observed no overall relations of lutein and zeaxanthin in the diet and serum to ARM in the overall NHANES III sample. The observation of relations of these dietary carotenoids that were limited to specific types of ARM lesions and specific segments of the population may reflect a protective role that may have been difficult to detect in the overall sample due to bias of these relations in older participants or a lack of vulnerability in non-White participants. Alternatively, the associations that were observed may be due to chance. This work supports the need for further research to evaluate the potential protective influence of dietary lutein and zeaxanthin on this common and visually debilitating condition of aging.

ACKNOWLEDGMENTS

Supported by National Institutes of Health grant EY11722 and awards to the Department of Ophthalmology and to Dr. Julie A. Mares-Perlman from the Research to Prevent Blindness.

The authors are grateful to Jillian N. Trabulsi for her work in computing estimates of carotenoid intake from responses to dietary questionnaires.

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