The Relationships between Macular Pigment Optical Density and Its Constituent Carotenoids in Diet and Serum

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Purpose. Lutein (L) and zeaxanthin (Z) are two dietary carotenoids that accumulate at the macula, where they are collectively known as macular pigment (MP). There is a biologically plausible rationale, with some supporting evidence, that MP may protect against age-related maculopathy (ARM). This study was undertaken to investigate the relationship between dietary intake of L and Z, serum concentrations of these carotenoids, and MP optical density in 828 healthy Irish subjects.

Methods. Dietary intake of L and Z was assessed with a validated food-frequency questionnaire, and serum concentrations of these carotenoids were quantified by high-performance liquid chromatography. MP optical density was measured psychophysically, using heterochromatic flicker photometry. Demographic data, lifestyle data, and general health status, were also recorded by questionnaire, with particular attention directed toward risk-factors (established and putative) for ARM.

Results. The relationships between MP optical density, serum concentrations of L (and Z), and dietary intake of L (and Z) were positive and statistically significant when analyzed for the entire study group ($r = 0.136–0.303$; $P < 0.01$ for all). Subjects with a clinically confirmed family history of ARM, current heavy cigarette smokers, subjects aged more than 53 years, and subjects with a body mass index (BMI) >27, did not demonstrate a positive and significant relationship between MP optical density and serum concentrations of Z ($r = 0.041$, $r = 0.001$, $r = 0.074$ and $r = 0.082$, respectively; $P > 0.05$ for all). However, there was a positive and significant relationship between MP optical density and serum concentrations of L in the presence of all these risk factors ($r = 0.165$ to 0.257), except for current heavy smokers ($r = 0.042$; $P > 0.05$).

Conclusions. For subjects at increased risk of ARM (e.g., subjects with a clinically confirmed family history of ARM, current heavy cigarette smokers, subjects aged > 53 years and subjects with a BMI > 27) retinal capture and/or retinal stabilization of Z appears to be compromised, whereas retinal uptake and/or stabilization of L appears to be compromised in current heavy smokers only. Given the lack of MP in association with risk for ARM, the findings indicate that a retina predisposed to this condition may have an impaired ability to accumulate circulating Z. (*Invest Ophthalmol Vis Sci.* 2007;48:571–582) DOI: 10.1167/iovs.06-0864

Age-related macular degeneration (AMD), the late stage of age-related maculopathy (ARM), is the leading cause of blindness in individuals more than 65 years of age in the Western World.[1,2] The etiological mechanisms underlying ARM continue to elude, but there is a growing body of evidence implicating oxidative stress and/or cumulative blue light damage in the process.[3] The carotenoids lutein (L) and zeaxanthin (Z), to the exclusion of all other carotenoids, are concentrated in the macula, where they are collectively referred to as macular pigment (MP).

MP is a blue-light filter at a prereceptorial level,[4] and demonstrates powerful antioxidant properties.[5] Consequently, it is believed that MP may protect against macular diseases attributable to oxidative stress, most notably ARM. The hypothesized protective effect of MP for ARM is rendered all the more provocative by its dietary origins.

Existing evidence in support of the view that MP protects against ARM is dominated by cross-sectional and epidemiologic reports. In brief, this evidence refers to parallels between risk for ARM and a relative lack of L and/or Z in the diet and/or serum and/or macula (SanGiovanni JP et al. *IOVS* 2004;45: ARVO E-Abstract 2242).[6,7]

Any protective effect of MP for ARM is premised on its defense against chronic and cumulative oxidative and/or photochemical damage, and, as such, would need to be exerted over a long period and decades before the onset of disease.[3] Given that a positive and significant relationship between MP and its constituent carotenoids in the diet and in the serum has been consistently demonstrated in healthy subjects[8–14] and that a relative lack of MP has been reported in association with certain risk-factors for ARM (SanGiovanni JP et al. *IOVS* 2004; 45:ARVO E-Abstract 2242),[6,7] it would seem logical to investigate whether the relationship between dietary and serum levels of these carotenoids, or whether the relationship between serum concentrations of L (and/or Z) and MP, is influenced by these risk-factors in young and middle-aged subjects. In other words, and for example, is tobacco use (an established risk-factor for ARM) associated with an attenuated relationship between serum levels of L and MP optical density? And if so, can the data indicate whether such a finding represents compromised retinal capture of circulating carotenoids among smokers or an altered stabilization/utilization of this xanthophyll within the retina of those who consume tobacco? To date, the influence of risk factors for ARM on these relationships has not been investigated, with the exception of the influence of sex.[15] However, that study reported the concentrations of L and Z collectively rather than individually.

The present study was designed to investigate the relationship between dietary intake of L and Z, serum concentrations of these carotenoids, and MP optical density, and to relate the findings to risk factors for ARM (both putative and established), in 828 healthy Irish subjects aged 20 to 60 years.

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Supported by Fighting Blindness Ireland.

Submitted for publication July 26, 2006; revised September 6, 2006; accepted November 22, 2006.

Disclosure: **J.M. Nolan**, None; **J. Stack**, None; **E. O'Connell**, None; **S. Beatty**, None

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METHODS

Subjects

Eight hundred twenty-eight healthy subjects from an Irish population were enrolled to participate in the study, which was authorized by the Research Ethics Committee of the Waterford Institute of Technology. Informed consent was obtained from each subject, and the experimental procedures adhered to the tenets of the Declaration of Helsinki. The inclusion criteria were white race and age between 20 and 60 years, and exclusion criteria comprised any ocular disease, visual acuity less than 6/18 in both eyes, or pregnancy.

Subjects were recruited by one of two means. The majority of subjects were recruited as a result of posters, newsletters, and word of mouth in the local community (group 1: $n = 646$). Also, patients attending the Department of Ophthalmology at Waterford Regional Hospital who had ARM (early and/or late) were encouraged to invite their offspring to participate (group 2: $n = 182$). For a subject recruited by posters and/or word of mouth who thought he or she had a direct family history of ARM (offspring of ARM sufferers), the medical records for that subject's appropriate parent were examined by a trained ophthalmologist, and if there was a positive family history of ARM, confirmatory documentation was secured, and that subject was placed into the group referred to as having a clinically confirmed family history of ARM. Of the 828 subjects who were recruited in total, we were unable to obtain MP measurements in 28 of these (coefficient of variation [CoV] >20%; $n = 20$; and visual acuity <6/18 in both eyes; $n = 8$). In addition, we failed to obtain dietary data from 2 individuals and serum data from 10 individuals. Also, demographic data from two individuals was misplaced.

Personal Details Questionnaire

Demographic data, lifestyle data, and general health status, were recorded by questionnaire, with particular attention directed toward risk factors (established and putative) for ARM. The risk factors we investigated included sex; family history of ARM (subjects with a positive family history of ARM had to have documented proof from the clinician who diagnosed the findings); cigarette smoking status (nonsmokers, current light smokers [<20 cigarettes per day], current heavy smokers [≥20 cigarettes per day]); age; body mass index (BMI; calculated as kg/m^2).

Dietary Analysis

A self-administered, semiquantitative food-frequency questionnaire (FFQ) developed by the Scottish Collaborative Group was used for dietary analysis. This previously validated FFQ[16,17] has been described in more detail elsewhere.[12] The questionnaire was designed to estimate a subject's normal diet over the previous 2 to 3 months, which included 166 commonly eaten food types or drink, grouped into 19 selections. The questionnaire was completed by the volunteer in the presence of the primary investigator (JN), and took between 25 and 35 minutes to complete.

The FFQs were scanned and verified by a trained dietary data coder using optical recognition software (Teleform Version 7; Cardiff Software, Vista, CA) at the Medical Research Council Human Nutrition Research (Cambridge, UK). Nutrient analysis was conducted using the Oracle Relational Database Management System (ver. 7; Redwood Shores, CA).[18] Dietary intake of L and Z was calculated using food composition data from UK, European, and U.S. data sources,[19,20] using standard principles or criteria for the matching of food items and standardized recipes or manufacturer's ingredient information where necessary.[21–23]

Of the 826 dietary questionnaires completed and analyzed, 72 were considered unreliable because they suggested levels of energy intake that were deemed physiologically unlikely (outside the ranges 800–3500 kcal/d for women or 1000–4000K cal/d for men).[24] This was somewhat surprising, given the close inspection, and frequent questioning performed by the investigator during the questionnaire process. However, removing these data from our analysis seemed to have no impact on our findings, and therefore we decided to include the data in our main analysis.

In addition to the absolute dietary values (milligrams per day) obtained for L and Z, we calculated nutrient density (milligrams per kilocalorie) and energy-adjusted (by residuals method) values of L and Z. These techniques have been described in Willet's *Nutritional Epidemiology*.[24]

Serum Analysis

Blood samples (6–8 mL) were collected from all subjects on the same day as the dietary and MP optical density analysis. Serum was separated from blood by centrifugation at 5000 rpm for 10 minutes and then aliquoted into three light-sensitive microcentrifuge tubes and stored at −70°C until time of analysis. Duplicate extractions were performed for each serum sample. A 0.4-mL aliquot of serum was pipetted into a light-sensitive microcentrifuge tubes (2 mL total capacity). Ethanol (0.30 mL) containing 0.25 g/L butyrated hydroxytoluene (BHT) and internal standard (tocopherol acetate) was added to each tube. Heptane (0.5 mL) was then added, and samples were vortexed vigorously for 1 minute followed by centrifugation at 2000 rpm for 5 minutes (MSC Micro Centaur; Davison & Hardy Ltd., Belfast, UK). The resultant heptane layer was retained and transferred to a second labeled light-sensitive microcentrifuge tube, and a second heptane extraction was performed. The combined heptane layers were immediately evaporated to dryness under nitrogen. These dried samples were reconstituted in methanol (200 μL), and 150 μL was injected for high-performance liquid chromatography (HPLC) analysis.

We used an HPLC system (Hewlett-Packard HP 1090 LC; Agilent, Dublin, Ireland) with photodiode array detection. A 5-μm analytical/preparative 4.6 × 250-mm specialized reversed-phase column (201TP; Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran. The flow rate was 1 mL/min, and the total run time was 15 minutes. All carotenoid peaks were integrated and quantified (Chem Station software; Agilent).

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards that were used to generate standard curves for quantification of these carotenoids. This assay was validated against the National Institute of Standards and Technology (NIST) Standard before analysis.

MP Optical Density Measurement

MP was measured psychophysically (Maculometer, developed by John Mellerio; University of Westminster, London, UK). This device utilizes the principle of heterochromatic flicker photometry (HFP), a technique that has been described in more detail elsewhere.[25,26] The Maculometer has been validated against motion photometry in normal subjects.[26]

The concentration of MP peaks at the center of the fovea and is optically undetectable at an eccentricity of 5°.[4] In a test field that alternates between blue light (470 nm, maximum MP absorption is at 460 nm) and green light (530 nm, not absorbed by MP), the luminance of the blue is varied until there is no or minimal flicker, which means the lights are isoluminant. Isoluminance matches are made with the test field at 0° (foveal) eccentricity and at 5.5° (parafoveal) eccentricity, so the logarithm of the ratio of the blue luminances in the fovea to that in the parafovea gives the MP optical density.

The flicker frequencies were fixed at 18 Hz for the foveal target and 13 Hz for the parafoveal target. The inability to customize flicker frequency for each subject is a limitation of the current device, as it may lead to increased uncertainty of the match endpoint. Subjects were allowed to make two or three trial minimum flicker matches before measurements were recorded. Six foveal readings were obtained, followed by six parafoveal readings.

Statistical Analysis

Commercial statistical software (SPSS; ver. 11, SPSS, Chicago, IL, and Statistica, ver. 7, StatSoft, Tulsa, OK) were used for analysis. Our main

TABLE 1. Pearson Correlation Matrix Showing Relationships between the Study Parameters for the Entire Study Group

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.963*	1							
Nutrient density of dietary L	0.848*	0.928*	1						
Serum L (μg/mL)	**0.280***	**0.303***	**0.299***	1					
Absolute dietary Z (mg/day)	0.697*	0.609*	0.479*	0.169*	1				
Energy-adjusted dietary Z	0.664*	0.668*	0.603*	0.206*	0.912*	1			
Nutrient density of dietary Z	0.585*	0.643*	0.677*	0.231*	0.805*	0.939*	1		
Serum Z (μg/mL)	0.160*	0.166*	0.146*	0.462*	**0.237***	**0.260***	**0.259***	1	
MP optical density	**0.208***	**0.182***	**0.136***	**0.181***	**0.218***	**0.184***	**0.166***	**0.166***	1

Bold font indicates relationships of major interest $n = 828$.

* Correlation is significant at the 0.01 level (Pearson correlation: two-tailed).

statistical methods included Pearson correlation testing, partial correlation testing, and one-way analysis of covariance (ANCOVA). Significance was set at the standard $P < 0.05$.

We performed power analyses for comparing subgroups (e.g., male/female, non–cigarette smokers, light cigarette smokers, and heavy cigarette smokers) for each of the outcome variables of interest: serum L, serum Z, and MP optical density. In each case, the difference we sought to detect was 30% of the overall mean for that outcome variable. In all but one case, the subgroup sizes were adequate to achieve statistical power of 0.90 or better (5% level of significance, two-tailed tests). The exception was the heavy cigarette-smoking subgroup, which had $n = 46$ subjects but would have required $n = 86$ subjects for 90% power for serum Z. However, given the known dose–response relationship between cigarette smoking and MP levels,[27] we feel we can justify investigating this important and interesting subgroup.

RESULTS

For this study, we report on right-eye MP optical density. However, for some subjects (15 of the total, 1.8%), only left-eye MP optical density was measured (as the right eye's visual acuity was <6/18 and therefore did not fit the required criteria for testing). For these subjects, we report the left-eye MP optical density.

There was a positive and significant relationship between MP optical density and each of its constituent carotenoids in the serum and in the diet (for absolute, energy-adjusted, and nutrient densities). Also, there was a positive and significant relationship between serum concentrations of L (and Z) and dietary intake (whether absolute, energy-adjusted, or nutrient density) and its respective carotenoid for the study group ($n = 828$; Pearson correlation: $r = 0.136–0.303$; $P < 0.01$, for all); Table 1. We also analyzed these relationships after removing subjects whose dietary energy intake was deemed unrealistic (see the Methods section; $n = 754$; Table 2), but this measure did not alter the strength or significance of the observed relationships, and we therefore elected to conduct the remainder of our analyses for the entire study group ($n = 828$).

The Relationship between Dietary L (and Z) and Serum L (and Z)

The relationship between dietary intake of L and Z (absolute, energy-adjusted and nutrient densities) and serum concentrations of the respective carotenoids were positive and statistically significant (Table 1). The strength of the relationships between absolute dietary intake of L (and Z) (milligrams per day) and serum L (and Z) (micrograms per milliliter) were slightly altered after adjusting for age, sex, family history status, smoking habits, dietary fat intake and BMI (partial correlation: $r = 0.309$, $P < 0.01$ and $r = 0.250$, $P < 0.01$, respectively).

Sex. The relationships between serum levels of L and Z and dietary intake (whether absolute, energy-adjusted, or nutrient

TABLE 2. Pearson Correlation Matrix Showing Relationships between the Study Parameters for the Entire Study Group after Removing Subjects with Unrealistic Energy Values

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.984*	1							
Nutrient density of dietary L	0.889*	0.938*	1						
Serum L (μg/mL)	**0.286***	**0.301***	**0.300***	1					
Absolute dietary Z (mg/day)	0.682*	0.637*	0.529*	0.189*	1				
Energy-adjusted dietary Z	0.658*	0.637*	0.615*	0.216*	0.953*	1			
Nutrient density of dietary Z	0.608*	0.669*	0.678*	0.238*	0.862*	0.951*	1		
Serum Z (μg/mL)	0.150*	0.645*	0.143*	0.455*	**0.249***	**0.254***	**0.258***	1	
MP optical density	**0.198***	**0.153***	**0.137***	**0.170***	**0.203***	**0.175***	**0.154***	**0.160***	1

Bold font indicates relationships of major interest. $n = 754$.

* Correlation is significant at the 0.01 level (Pearson correlation: two-tailed).

TABLE 3. Pearson Correlation Matrix Showing Relationships between the Study Parameters, Analyzed Separately for Men and Women

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Men (n = 288)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.937*	1							
Nutrient density of dietary L	0.871*	0.950*	1						
Serum L (μg/mL)	**0.201***	**0.300***	**0.263***	1					
Absolute dietary Z (mg/day)	0.651*	0.554*	0.521*	0.136*	1				
Energy-adjusted dietary Z	0.560*	0.598*	0.586*	0.147*	0.926*	1			
Nutrient density of dietary Z	0.464*	0.529*	0.578*	0.133*	0.846*	0.950*	1		
Serum Z (μg/mL)	0.201*	0.217*	0.178*	0.448*	**0.293***	**0.319***	**0.295***	1	
MP optical density	**0.261***	**0.234***	**0.194***	**0.195***	**0.236***	**0.206***	**0.165***	**0.164***	1
Women (n = 538)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.955*	1							
Nutrient density of dietary L	0.854*	0.938*	1						
Serum L (μg/mL)	**0.271***	**0.292***	**0.299***	1					
Absolute dietary Z (mg/day)	0.718*	0.602*	0.476*	0.179*	1				
Energy-adjusted dietary Z	0.658*	0.688*	0.624*	0.219*	0.874*	1			
Nutrient density of dietary Z	0.633*	0.681*	0.699*	0.255*	0.797*	0.946*	1		
Serum Z (μg/mL)	0.139*	0.139*	0.133*	0.449*	**0.211***	**0.230***	**0.241***	1	
MP optical density	**0.207***	**0.196***	**0.167***	**0.195***	**0.221***	**0.217***	**0.205***	**0.176***	1

Bold font indicates relationships of major interest.

* Correlation is significant at the 0.01 level (Pearson correlation: two-tailed).

density) of the respective carotenoids remained positive and significant when the data were analyzed separately for the men (n = 288, 35%) and women (n = 538, 65%; Table 3). After controlling for absolute dietary intake of L, the adjusted group mean for serum L was significantly higher in the women than in the men (ANCOVA, $P = 0.048$; Fig. 1), whereas the adjusted group mean for serum Z was statistically similar for the two sexes (ANCOVA, $P > 0.05$; Fig. 2). However, when we controlled for other variables, in addition to dietary L (e.g., age, BMI) any differences in serum L between males and females were no longer significant. The observed significant difference for gender displayed in Figure 1 is most likely attributable therefore to confounding variables such as cigarette smoking and BMI.

Family History Status. The respective relationships between serum concentrations of L and Z and dietary intake (whether absolute, energy-adjusted, or nutrient density) of these carotenoids remained positive and significant when the data were analyzed separately for subjects with (n = 182, 22%) and without (n = 644, 78%) a clinically confirmed family history of ARM (Table 4). However, after controlling for absolute dietary intake of L, the adjusted group mean for serum L was significantly higher for offspring of those with ARM than for subjects without such a family history (ANCOVA, $P < 0.01$; Fig. 1), whereas the adjusted group mean for serum Z was statistically similar for these subgroups (ANCOVA, $P > 0.05$; Fig. 2).

Cigarette Smoking. Of the 826 subjects with smoking information available, 660 (80%) were nonsmokers, 120 (14.5%) smoked <20 cigarettes per day, and 46 (5.5%) smoked >20 cigarettes each day. The relationships between serum concentrations of L and Z and dietary intake (whether absolute, energy-adjusted or nutrient density) of the respective carotenoids remained positive and significant when the data were analyzed separately for nonsmokers and current light smokers (<20 cigarettes per day; Table 5).

There was a positive and significant relationship between dietary Z and serum Z for current heavy smokers (≥20 cigarettes per day, n = 46, 5.5%), whereas dietary L was not significantly related to serum L in this group (Table 5). Also, the mean adjusted serum L concentration (controlling for absolute dietary intake of L) was significantly higher for nonsmokers when compared to either light or heavy current smokers (ANCOVA, $P < 0.01$; Fig. 1). The adjusted group mean for serum Z (controlling for absolute dietary intake of this carotenoid) was lower for heavy current smokers when compared with

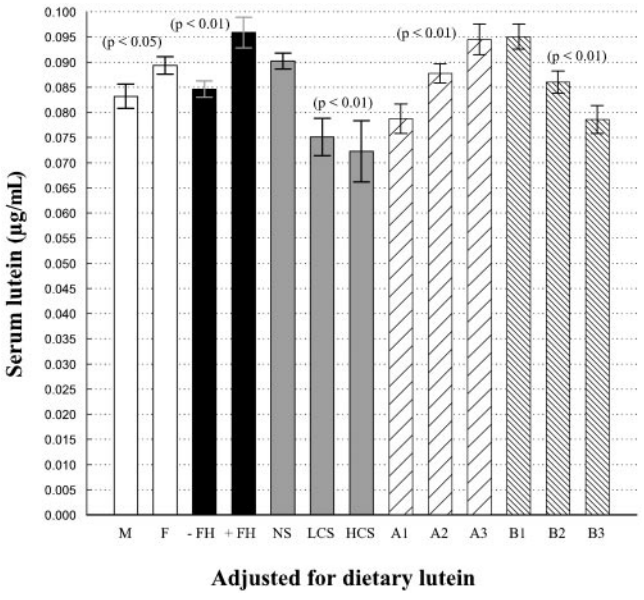

FIGURE 1. Comparative representation of mean serum lutein concentrations, adjusted for differences in absolute dietary intake of lutein, with respect to variables relevant to risk for age-related maculopathy (ANCOVA); *vertical bars* denote ± 1.96 SE. M, male; F, female; −FH, subjects with no known family history of ARM; +FH, subjects with a clinically confirmed family history of ARM; NS, nonsmokers; LCS, light current smokers (<20 cigarettes per day); HCS, heavy current smokers (≥20 cigarettes per day); A1, subjects aged <31 years; A2, subjects aged between 31 and 53 years; A3, subjects aged >53 years; B1, BMI < 23; B2, BMI between 23 and 27; B3, BMI > 27.

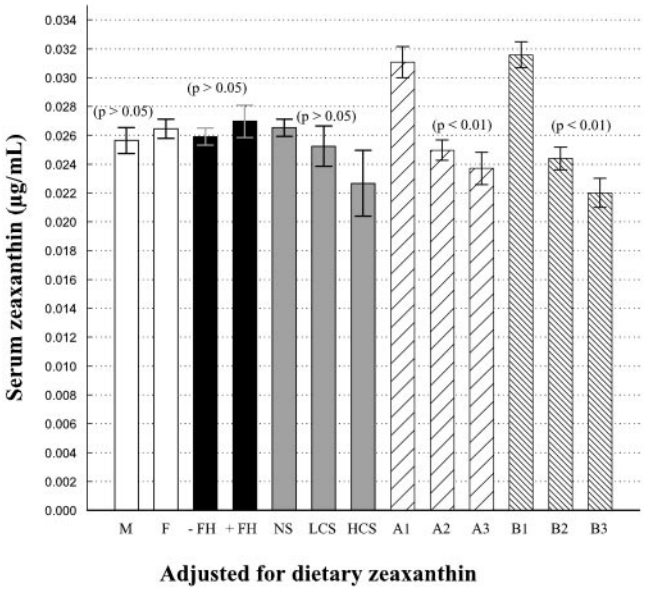

FIGURE 2. Comparative representation of mean serum zeaxanthin concentrations, adjusted for differences in absolute dietary intake of zeaxanthin, with respect to variables relevant to risk for age-related maculopathy (ANCOVA); *vertical bars* denote ± 1.96 SE. Abbreviations are as in Figure 1.

light current smokers or nonsmokers, but the differences were not statistically significant (ANCOVA, $P > 0.05$; Fig. 2).

Age. We investigated the relationships between serum concentrations of L and Z and dietary intake (absolute, energy-adjusted, and nutrient densities) of the respective carotenoids, and found the relationships to be of equal significance for subjects aged <31 years, between 31 and 53 years, and >53 years (Table 6). Subjects in the upper age tertile (>53 years) had a significantly higher mean adjusted serum L concentration (controlling for absolute dietary intake of L) than did subjects in the middle (aged 31–53 years) and lower (<31 years) age tertiles (ANCOVA, $P < 0.01$; Fig. 1). In contrast, subjects in the lower age tertile had a significantly higher mean adjusted serum Z concentration (controlling for absolute dietary intake of Z) than did subjects in the middle and higher age tertiles (ANCOVA, $P < 0.01$; Fig. 2).

Body Mass Index. The relationships between serum concentration of L and Z and dietary intake (absolute, energy-adjusted, and nutrient density) of the respective carotenoids were positive and significant for subjects with a BMI of <23, between 23 and 27, and >27 (Table 7). However, subjects with a BMI of <23 had significantly higher adjusted mean serum L (and Z; having controlled for absolute dietary intake of these carotenoids separately and respectively for these analyses) than subjects in the middle and upper BMI tertiles (BMI = 23–27 and BMI > 27, respectively; ANCOVA, $P < 0.01$, for all; Figs. 1, 2).

The Relationship between Serum L (and Z) and MP Optical Density

The relationships between MP optical density and serum concentrations of each of its constituent carotenoids was positive and significant (Table 1). The strength of the relationship between serum L and MP optical density was improved after adjusting for age, sex, family history, smoking status, and absolute dietary intake of L (partial correlation: $r = 0.232$, $P < 0.01$), whereas the strength of the relationship between serum Z and MP optical density was reduced after controlling for the

TABLE 4. Pearson Correlation Matrix Showing Relationships between the Study Parameters, Analyzed Separately for Subjects with and without a Clinically Confirmed Family History of ARM

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (µg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (µg/mL)	MP Optical Density
No known family history of ARM ($n = 644$)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.962*	1							
Nutrient density of dietary L	0.861*	0.944*	1						
Serum L (µg/mL)	**0.239***	**0.251***	**0.241***	1					
Absolute dietary Z (mg/day)	0.684*	0.595*	0.473*	0.154*	1				
Energy-adjusted dietary Z	0.619*	0.642*	0.584*	0.169*	0.916*	1			
Nutrient density of dietary Z	0.538*	0.592*	0.604*	0.182*	0.821*	0.949*	1		
Serum Z (µg/mL)	0.141*	0.141*	0.118*	0.492*	**0.241***	**0.256***	**0.259***	1	
MP optical density	**0.230***	**0.199***	**0.174***	**0.210***	**0.228***	**0.186***	**0.174***	**0.209***	1
Clinically confirmed family history of ARM ($n = 182$)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.996*	1							
Nutrient density of dietary L	0.842*	0.916*	1						
Serum L (µg/mL)	**0.364***	**0.413***	**0.394***	1					
Absolute dietary Z (mg/day)	0.748*	0.660*	0.526*	0.210*	1				
Energy-adjusted dietary Z	0.723*	0.751*	0.681*	0.308*	0.900*	1			
Nutrient density of dietary Z	0.708*	0.769*	0.836*	0.344*	0.764*	0.916*	1		
Serum Z (µg/mL)	0.231*	0.260*	0.242*	0.377*	**0.216***	**0.279***	**0.266***	1	
MP optical density	**0.240***	**0.221***	**0.149†**	**0.257***	**0.228***	**0.205***	**0.167†**	**0.041‡**	1

Bold font indicates relationships of major interest.

* Correlation is significant at the 0.01 level (Pearson correlation: two-tailed).

† Correlation is significant at the 0.05 level (Pearson correlation: two-tailed).

‡ Nonsignificant relationships.

TABLE 5. Pearson Correlation Matrix Showing Relationships between the Study Parameters, Analyzed According to Smoking Status

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Nonsmokers (n = 660)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.966*	1							
Nutrient density of dietary L	0.858*	0.932*	1						
Serum L (μg/mL)	**0.265***	**0.283***	**0.282***	1					
Absolute dietary Z (mg/day)	0.681*	0.595*	0.482*	0.173*	1				
Energy-adjusted dietary Z	0.634*	0.658*	0.606*	0.205*	0.911*	1			
Nutrient density of dietary Z	0.585*	0.637*	0.677*	0.231*	0.813*	0.944*	1		
Serum Z (μg/mL)	0.103*	0.108*	0.103*	0.437*	**0.218***	**0.241***	**0.248***	1	
MP optical density	**0.200***	**0.178***	**0.145***	**0.165***	**0.211***	**0.182***	**0.167***	**0.165***	1
Current light smokers <20 cigarettes per day (n = 120)									
Energy-adjusted dietary L	0.958*	1							
Nutrient density of dietary L	0.896*	0.912*	1						
Serum L (μg/mL)	**0.431***	**0.488***	**0.488***	1					
Absolute dietary Z (mg/day)	0.756*	0.665*	0.477*	0.211*	1				
Energy-adjusted dietary Z	0.679*	0.701*	0.595*	0.268*	0.920*	1			
Nutrient density of dietary Z	0.554*	0.628*	0.653*	0.293*	0.770*	0.920*	1		
Serum Z (μg/mL)	0.406*	0.426*	0.385*	0.501*	**0.300***	**0.320***	**0.297***	1	
MP optical density	**0.323***	**0.311***	**0.188†**	**0.224***	**0.304***	**0.294***	**0.226***	**0.194†**	1
Current heavy smokers ≥20 cigarettes per day (n = 46)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.941*	1							
Nutrient density of dietary L	0.862*	0.961*	1						
Serum L (μg/mL)	**0.154‡**	**0.153‡**	**0.154‡**	1					
Absolute dietary Z (mg/day)	0.766*	0.677*	0.569*	0.085	1				
Energy-adjusted dietary Z	0.678*	0.745*	0.688*	0.079	0.897*	1			
Nutrient density of dietary Z	0.704*	0.289*	0.752*	0.101	0.873*	0.983*	1		
Serum Z (μg/mL)	0.275*	0.426*	0.263*	0.674*	**0.291***	**0.313***	**0.308***	1	
MP optical density	**0.019‡**	**−0.134‡**	**−0.165‡**	**0.042‡**	**0.094‡**	**−0.104‡**	**−0.138‡**	**0.001‡**	1

Symbols and significances are as described in Table 4.

same variables (and replacing absolute dietary intake of L with that of Z; partial correlation: $r = 0.082$, $P = 0.025$).

Sex. The relationship between MP optical density and serum concentrations of L (and Z) remained positive and statistically significant when the data were analyzed separately for the men and women (Table 3). However, after adjustment for serum L (and Z, respectively), the adjusted group mean for MP optical density was significantly higher in the men than in the women (ANCOVA, $P < 0.01$, for both; Figs. 3, 4).

Family History. The relationship between MP optical density and serum concentrations of L remained positive and significant for subjects with and without a clinically confirmed family history of ARM (Table 4), on separate analysis of the data for these two subgroups. However, there was no statistically demonstrable relationship between MP optical density and serum concentrations of Z in subjects who had a clinically confirmed family history of ARM, whereas a positive and significant relationship between MP optical density and serum levels of Z was demonstrable in subjects who reported having no known family history of this condition (Table 4, Fig. 5).

Also, after controlling for serum L (and separately for serum Z), the adjusted group mean for MP optical density was significantly higher for subjects with no known family history of ARM when compared to subjects with a clinically confirmed family history of disease (ANCOVA, $P < 0.01$, for both; Figs. 3, 4).

Cigarette Smoking. The relationship between MP optical density and serum concentrations of L and Z remained positive and significant for nonsmokers and for light current smokers, when the data were analyzed separately for these two subgroups (Table 5; Figs. 6, 7). However, there was no statistically demonstrable relationship between MP optical density and serum concentrations of L or Z among heavy current smokers (Table 5; Figs. 6, 7). Also, mean adjusted MP optical density (controlling for serum L and Z, respectively) was significantly lower for current heavy smokers when compared to either current light smokers or to nonsmokers (ANCOVA, $P < 0.01$; Figs. 3, 4).

Age. We investigated the relationships between MP optical density and serum concentrations of L (and Z) for subjects aged <31 years, between 31 and 53 years, and >53 years (Table 6). There was no statistically demonstrable relationship between serum concentrations of Z and MP optical density in subjects aged >53 years ($r = 0.074$, $P > 0.05$), in contrast to relationships between serum concentration of this carotenoid and MP optical density in subjects aged <31 years and in those aged between 31 and 53 years, which were positive and significant ($r = 0.197$ and $r = 0.137$, respectively; $P < 0.05$, for both). Also, subjects in the upper age tertile (>53 years) had a statistically significant lower mean adjusted MP optical density (controlling for serum L and Z, respectively) than did subjects in the middle (aged 31–53 years) and lower (<31 years) age tertiles (ANCOVA, $P < 0.01$; Figs. 3, 4).

Body Mass Index. There was no statistically demonstrable relationship between MP optical density and serum concentrations of Z in subjects with BMI > 27 ($r = 0.082$, $P > 0.05$), in contrast with the positive and significant relationships seen in subjects with a BMI < 23 and those with BMI between 23 and 27 ($r = 0.230$ and $r = 0.194$, respectively; $P < 0.01$, for both; Table 7). The relationship between MP optical density and serum concentrations of L was positive and significant, irre-

TABLE 6. Pearson Correlation Matrix Showing Relationships between Study Parameters, Analyzed Separately for Subjects <31 Years, between 31 and 53 Years, and >53 Years

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Subjects aged <31 years (n = 195)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.966*	1							
Nutrient density of dietary L	0.871*	0.943*	1						
Serum L (μg/mL)	**0.310***	**0.333***	**0.313***	1					
Absolute dietary Z (mg/day)	0.762*	0.687*	0.542*	0.206*	1				
Energy-adjusted dietary Z	0.715*	0.735*	0.639*	0.239*	0.931*	1			
Nutrient density of dietary Z	0.648*	0.702*	0.667*	0.250*	0.847*	0.951*	1		
Serum Z (μg/mL)	0.102*	0.134*	0.138*	0.513*	**0.191***	**0.210***	**0.226***	1	
MP optical density	**0.170†**	**0.157***	**0.153†**	**0.233***	**0.147***	**0.133†**	**0.233***	**0.197***	1
Subjects aged 31 to 53 years (n = 435)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.962*	1							
Nutrient density of dietary L	0.844*	0.925*	1						
Serum L (μg/mL)	**0.295***	**0.307***	**0.311***	1					
Absolute dietary Z (mg/day)	0.665*	0.576*	0.469*	0.164*	1				
Energy-adjusted dietary Z	0.610*	0.645*	0.607*	0.185*	0.899*	1			
Nutrient density of dietary Z	0.569*	0.633*	0.701*	0.223*	0.790*	0.933*	1		
Serum Z (μg/mL)	0.160*	0.226*	0.144*	0.473*	**0.241***	**0.257***	**0.253***	1	
MP optical density	**0.239***	**0.237***	**0.192***	**0.237***	**0.233***	**0.193***	**0.160***	**0.137†**	1
Subjects aged >53 years (n = 196)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.970*	1							
Nutrient density of dietary L	0.866*	0.941*	1						
Serum L (μg/mL)	**0.260***	**0.283***	**0.262***	1					
Absolute dietary Z (mg/day)	0.693*	0.614*	0.490*	0.247*	1				
Energy-adjusted dietary Z	0.628*	0.646*	0.596*	0.293*	0.917*	1			
Nutrient density of dietary Z	0.548*	0.597*	0.621*	0.294*	0.818*	0.951*	1		
Serum Z (μg/mL)	0.249*	0.266*	0.249*	0.563*	**0.252***	**0.288***	**0.305***	1	
MP optical density	**0.189***	**0.198***	**0.195***	**0.177†**	**0.198***	**0.224***	**0.233***	**0.074‡**	1

Symbols and significances are as described in Table 4.

spective of BMI status (Table 7). Mean adjusted MP optical density (controlling for serum L and Z, respectively) was statistically comparable in the lower (BMI < 23), middle (BMI = 23–27), and upper (BMI > 27) BMI tertiles (ANCOVA, $P > 0.05$, for all; Figs. 3, 4).

DISCUSSION

This study was specifically designed to report on the relationships between dietary intake of L and Z, serum concentrations of L and Z, and MP optical density, and to relate the findings to risk for ARM in 828 healthy Irish subjects aged 20 to 60 years.

Of the eight published observational studies that have examined the relationship between dietary antioxidants and risk for ARM, four have found a protective effect associated with a high intake of carotenoids.[7,28–35] Also, a recent report by the Age-Related Eye Disease Study group has shown that a higher dietary intake of L and Z is associated with a decreased likelihood of the development of advanced ARM (SanGiovanni JP et al. *IOVS* 2004;45:ARVO E-Abstract 2242).

Mean daily intake of L and Z combined, varies from 0.8 mg to 4 mg per day, depending on the population studied and the method of dietary assessment used.[36,37] Further, daily intake of L has been shown to vary widely among individuals, as illustrated by a standard deviation of 2.45 mg/d in a recently published study.[38] In the 826 volunteers who completed FFQs in our study, mean dietary intake (±SD) of L and Z was 1.399 ± 0.79 mg/d and 0.199 ± 0.117 mg/d, respectively. These values are comparable with those obtained by previous investigators (using similar dietary assessment techniques) in similar age groups.[10,11,27,39–42]

Most previous studies in which the relationship between serum concentrations of L (and Z) and risk for ARM have been examined, have reported the concentrations of these two carotenoids collectively rather than individually.[6,7,43–49] Of these, only one found a significant association between low serum concentrations of L and Z (combined) and risk for ARM.[46] Recently, however, Gale et al.[6] investigated the relationship between serum L and Z (separately) and risk for ARM and found that ARM was associated with a relative lack of serum Z (but not L). It is possible that the analysis of serum L and Z in a collective fashion in those previous studies, rather than investigating these carotenoids separately, had masked the relative importance of serum Z concentrations. In our study, mean serum levels (±SD) of L and Z were 0.087 ± 0.042 μg/mL and 0.026 ± 0.016 μg/mL, respectively, which are consistent with those obtained by previous investigators for similar age groups.[10,47,50–52]

Of the nine observational studies analyzing the relationship between dietary intake of L and Z and serum concentrations of these carotenoids, all have demonstrated significant and positive relationships (r = 0.21–0.74; $P < 0.05$ for all).[10–13,15,40–42,53] The largest of these studies included 2786 subjects, and found that every 10% increase in estimated dietary intake of L and Z was associated with a 2.4% increase in serum L concentration.[16] Similarly, we found a positive and significant relationship between the absolute dietary intake of L and Z, and serum concentrations of the respective carotenoids (r = 0.280 and 0.237, respectively).

TABLE 7. Pearson Correlation Matrix Showing Relationships between Study Parameters, Analyzed for Subjects with a BMI <23, with a BMI between 23 and 27, and with a BMI >27

	Absolute Dietary L (mg/day)	Energy-Adjusted Dietary L	Nutrient Density of Dietary L	Serum L (μg/mL)	Absolute Dietary Z (mg/day)	Energy-Adjusted Dietary Z	Nutrient Density of Dietary Z	Serum Z (μg/mL)	MP Optical Density
Subjects with BMI <23 (n = 270)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.970*	1							
Nutrient density of dietary L	0.834*	0.913*	1						
Serum L (μg/mL)	**0.302***	**0.325***	**0.294***	1					
Absolute dietary Z (mg/day)	0.781*	0.708*	0.526*	0.204*	1				
Energy-adjusted dietary Z	0.734*	0.751*	0.640*	0.237*	0.930*	1			
Nutrient density of dietary Z	0.664*	0.720*	0.724*	0.263*	0.814*	0.940*	1		
Serum Z (μg/mL)	0.151†	0.154*	0.103*	0.469*	**0.209***	**0.225***	**0.220***	1	
MP optical density	**0.317***	**0.278***	**0.194***	**0.181***	**0.358***	**0.309***	**0.250***	**0.230***	1
Subjects with BMI 23–27 (n = 345)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.959*	1							
Nutrient density of dietary L	0.830*	0.920*	1						
Serum L (μg/mL)	**0.320***	**0.349***	**0.351***	1					
Absolute dietary Z (mg/day)	0.699*	0.596*	0.463*	0.159*	1				
Energy-adjusted dietary Z	0.640*	0.659*	0.598*	0.201*	0.906*	1			
Nutrient density of dietary Z	0.595*	0.649*	0.695*	0.247*	0.800*	0.940*	1		
Serum Z (μg/mL)	0.189†	0.191†	0.164*	0.411*	**0.238***	**0.257***	**0.253***	1	
MP optical density	**0.193†**	**0.157†**	**0.113†**	**0.198†**	**0.164***	**0.112†**	**0.155†**	**0.194†**	1
Subjects with BMI >27 (n = 211)									
Absolute dietary L (mg/day)	1								
Energy-adjusted dietary L	0.959*	1							
Nutrient density of dietary L	0.881*	0.957*	1						
Serum L (μg/mL)	**0.171***	**0.179***	**0.199***	1					
Absolute dietary Z (mg/day)	0.578*	0.492*	0.428*	0.11	1				
Energy-adjusted dietary Z	0.513*	0.561*	0.553*	0.123	0.890*	1			
Nutrient density of dietary Z	0.437*	0.502*	0.553*	0.127	0.810*	0.946*	1		
Serum Z (μg/mL)	0.118*	0.133*	0.185*	0.484*	**0.217***	**0.249***	**0.273***	1	
MP optical density	**0.136†**	**0.112‡**	**0.083‡**	**0.165***	**0.201***	**0.168***	**0.155***	**0.082‡**	1

Symbols and significances are as described in Table 4.

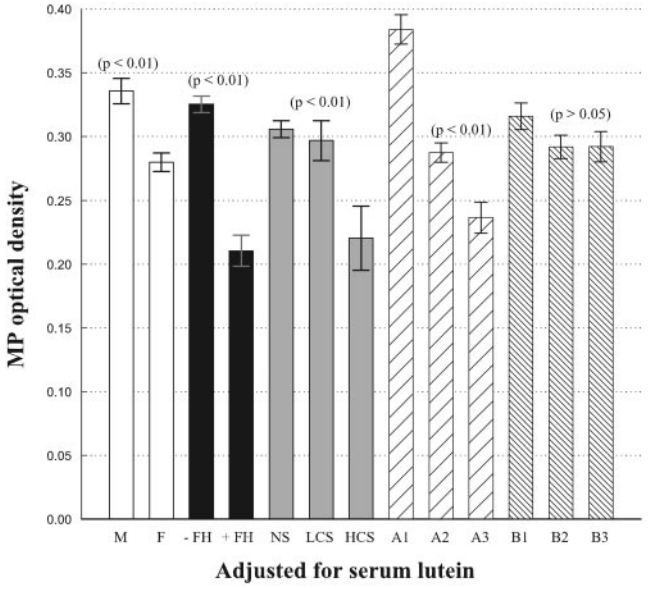

FIGURE 3. Comparative representation of MP optical density, adjusted for serum lutein concentrations, with respect to variables relevant to risk for age-related maculopathy (ANCOVA); *vertical bars*: ±SE. Abbreviations are as in Figure 1.

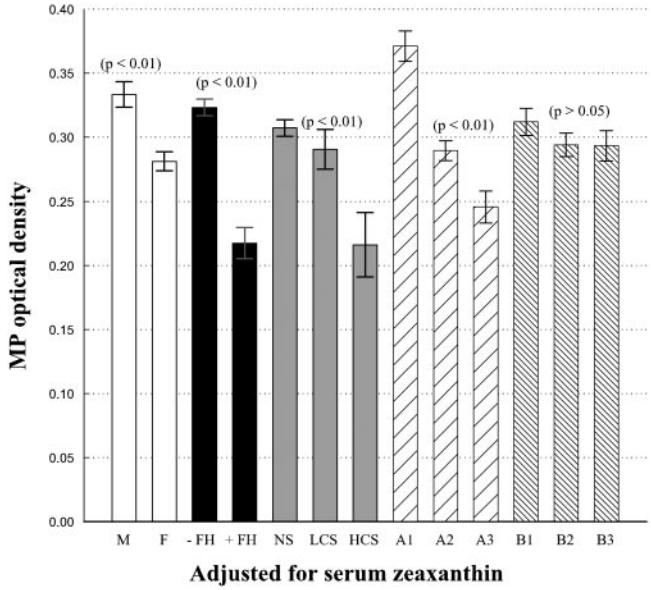

FIGURE 4. Comparative representation of MP optical density, adjusted for serum zeaxanthin concentrations, with respect to variables relevant to risk for age-related maculopathy (ANCOVA); *vertical bars*: ± SE. Abbreviations are as in Figure 1.

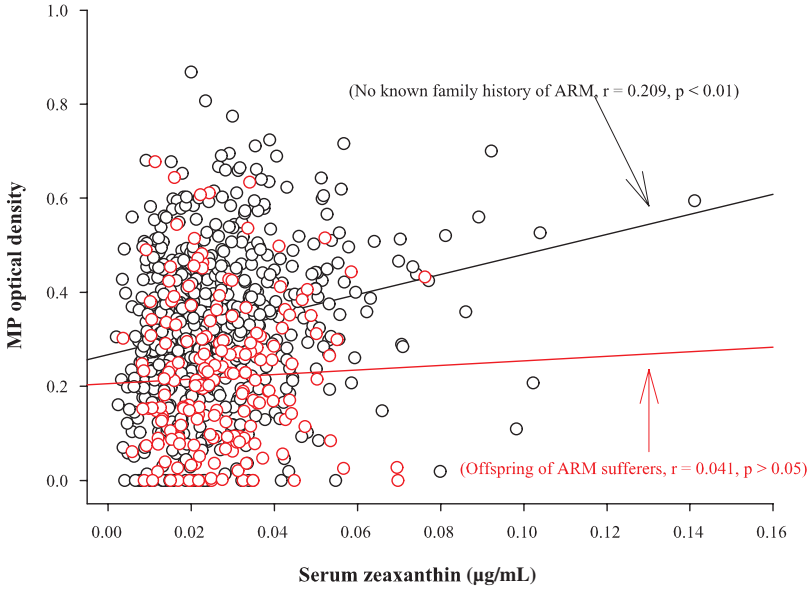

FIGURE 5. Relationship between serum concentrations of zeaxanthin (micrograms per milliliter) and MP optical density in 615 healthy subjects with no known family history of ARM and 176 healthy subjects with a clinically confirmed family history of ARM. MP, macular pigment.

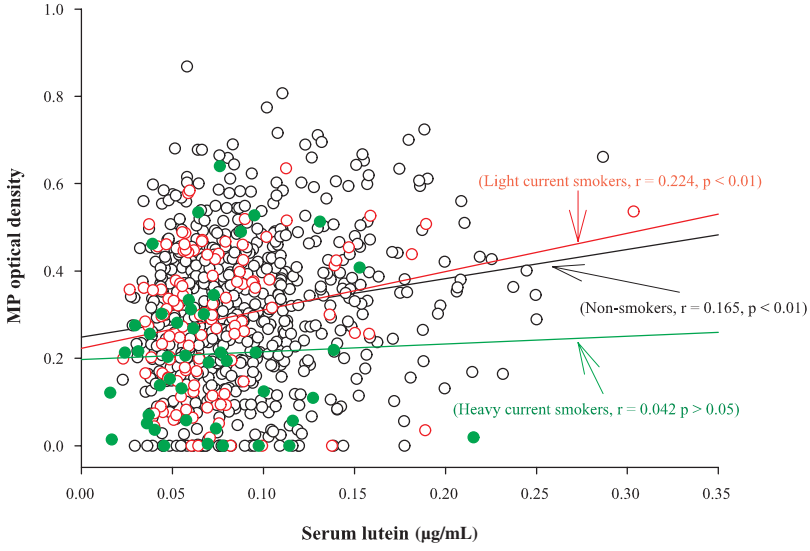

FIGURE 6. Relationship between serum concentrations of lutein (micrograms per milliliter) and MP optical density in 659 healthy subjects who were nonsmokers, 120 healthy subjects who were light current smokers (<20 cigarettes per day), and 45 healthy subjects who were heavy current smokers (≥20 cigarettes per day). MP, macular pigment.

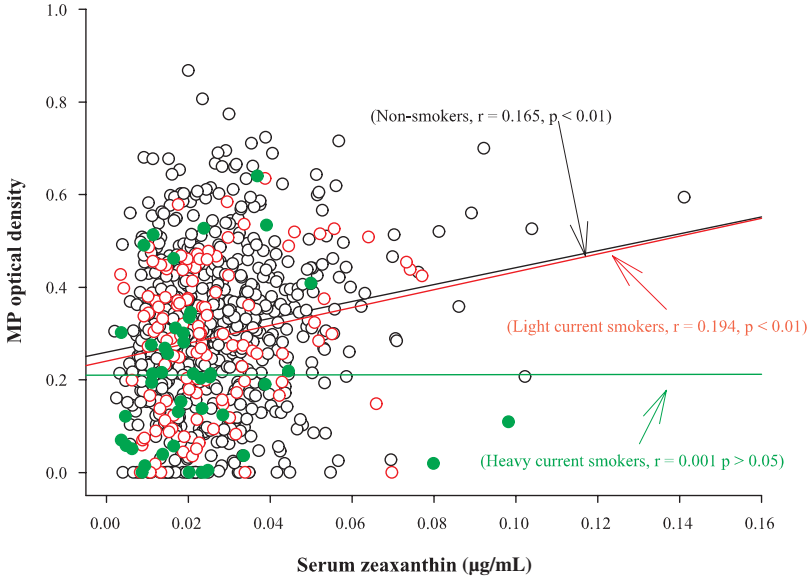

FIGURE 7. Relationship between serum concentrations of zeaxanthin (μg/mL) and MP optical density for 659 healthy subjects who were nonsmokers, 120 healthy subjects who were light current smokers (<20 cigarettes per day) and 45 healthy subjects who were heavy current smokers (≥20 cigarettes per day). MP, macular pigment.

Of note, after adjustment for confounding variables, the relationships were only slightly enhanced (absolute diet L/serum L, $r = 0.284$ and absolute diet Z/serum Z, $r = 0.250$).

Also, energy-adjusted (by the residuals method) and nutrient density L (and Z) were positively and significantly related to serum concentrations of their respective carotenoid, with Pearson correlation coefficients ranging from 0.259 to 0.303 for the entire study group. We felt it appropriate to calculate energy-adjusted values, as an absolute amount of a specific nutrient tends to have less of an effect for a larger, higher energy-consuming person, than for a smaller person. The slightly stronger relationships we found for energy-adjusted and nutrient density values most likely reflect this.

Further, the present study comprised a sample size large enough to assess and compare, for the first time, the relationships between dietary intake of L (and Z), serum concentrations of L (and Z) and MP optical density, among different groups in the normal population (e.g., males, females, subjects with a confirmed family history of ARM, subjects with no known family history of ARM, cigarette smokers, nonsmokers).

Of interest, subdividing according to sex, family history, cigarette smoking, age, and BMI (putative and known risk factors for ARM), the respective relationships between dietary intake and serum concentrations of L (and Z) remained positive and statistically significant, with the sole exception of the relationship between dietary (whether absolute, energy-adjusted or nutrient densities) and serum levels of L for current heavy smokers (≥20 cigarettes per day), who failed to exhibit a significant relationship between these measures (Table 5).

It appears, therefore, that the risk that sex, family history of ARM, smoking, age, and BMI represent for ARM is not attributable to impaired digestion and/or intestinal absorption of Z. Similarly, the risk that sex, family history of ARM, age, and BMI represent for ARM is not associated with impaired digestion or intestinal absorption of L. However, the failure of heavy cigarette smokers to exhibit the typical positive and significant relationship between dietary intake of L and serum concentrations of this carotenoid is interesting and warrants discussion.

Given that heavy cigarette smokers typically had a dietary intake of L similar to that of light cigarette smokers and nonsmokers ($P > 0.05$, for all), and that heavy cigarette smokers had lower serum levels of L than did nonsmokers (even after adjustment for dietary intake of L), indicates that heavy users of tobacco have a compromised digestion and/or absorption of this carotenoid or that consumption of cigarettes reduces circulating levels of L.

Possible mechanisms whereby tobacco use is related to reduced circulation of serum L and/or an impaired diet L/serum L relationship requires discussion. One possibility includes an altered lipoprotein profile in cigarette smokers, with a consequential impact on the transport of L within serum. For example, high-density lipoproteins (HDL) are known to be the primary carriers of L and Z, whereas low-density lipoproteins (LDL) transport hydrocarbon carotenoids (e.g., lycopene, β-carotene).[54] Given that cigarette smokers are known to have significantly reduced levels of HDL when compared to nonsmokers,[55] it is possible that reduced HDL in tobacco users may account for the reduced serum concentrations of L (and Z) that we report for such individuals. Alternatively, the increased circulating pro-oxidant load in cigarette smokers could result in depletion of circulating levels of L (and Z). Indeed, Handelman et al.[56] have shown that exposing human plasma to the gas phase of cigarette smoke results in a significant depletion (up to 60%) of L (+Z) after just 9 hours' exposure time (9 hours' exposure time = 3 puffs/h for 9 hours).

Studies investigating the relationship between retinal and serum carotenoids should be interpreted with caution, primarily because serum concentrations of L and Z reflect recent nutritional intake only.[57] In contrast, MP has a slow biological turnover, and probably reflects the local balance between pro-oxidant stresses and antioxidant defenses in the retina. In other words, a dramatic change in diet is unlikely to affect MP for several weeks, but will be reflected in much more rapid changes in serum concentrations of L and Z.

Of the nine observational studies investigating the relationship between serum L and Z and MP optical density, seven have found positive and significant correlations ($r = 0.21$–0.82, $P < 0.05$).[10,12,13,15,39,42,50,58,59] Of the studies that did not report significant relationships, one was limited by its inclusion of only 20 subjects (10 monozygotic twin pairs)[59] and the other by its narrow range of serum concentrations of L and Z, represented by standard deviations of 0.043 μg/mL and 0.014 μg/mL, respectively.[58]

We also found serum concentrations of L and Z to be positive and significant predictors of MP optical density. Of interest, controlling for age, sex, family history, cigarette smoking, and absolute dietary intake of L, enhanced the strength of the relationship between serum L and MP optical density ($r = 0.232$, $P < 0.01$), whereas, the strength of the relationship between serum Z and MP optical density was reduced after controlling for the same variables (and replacing absolute dietary intake of L with that of Z; $r = 0.082$, $P < 0.05$).

Finally, and of noteworthy interest, subjects with a clinically confirmed family history of ARM, current heavy cigarette smokers, subjects >53 years of age, and subjects with a BMI > 27, did not exhibit a significant relationship between MP optical density and serum concentrations of Z ($r = 0.041$, $r = 0.001$, $r = 0.074$ and $r = 0.082$, respectively, $P > 0.05$, for all). Similarly, current heavy cigarette smokers did not exhibit a positive and significant relationship between serum L and MP optical density ($r = 0.042$, $P > 0.05$). For all other subgroups (men, women, subjects with no known family history of ARM, nonsmokers, current light smokers, subjects aged <31 years, subjects between 31 and 52 years of age, subjects with a BMI <23, and subjects with a BMI between 23 and 27), the relationship between MP optical density and serum concentrations of L (and Z) were positive and statistically significant.

It appears, therefore, that risk factors for ARM are associated with a lack of the typical positive and significant relationship between serum concentrations of Z and MP optical density before the onset of disease. Although many of these risk factors were associated with lower levels of Z in serum and with lower MP optical density, it is the attenuated relationship between these variables that is of interest, and warrants discussion. The lack of a positive and significant relationship between serum Z and MP optical density could be attributable to defective capture of this carotenoid by the retina and/or impaired stabilization within the retina, in association with risk for ARM. Certainly, high BMI could be associated with competition between the retina and adipose tissue for circulating carotenoids, but it is difficult to explain why uptake of Z (and not L) by the retina may be compromised in association with other risk factors for ARM.

However, the mechanisms governing retinal capture of carotenoids remain poorly understood, and xanthophyll-binding proteins (XBPs) are thought to be important.[60] Of interest, a zeaxanthin-binding protein (ZBP; Pi isoform of glutathione S-transferase [GSTP1]), which is found in high concentrations of the inner and outer plexiform layers of the macula (location of MP),[4,61] has been shown to demonstrate a high affinity for dietary Z, with poor affinity for L.[62] This finding indicates that L and Z have unique specific binding proteins within the macula, which are responsible for uptake and/or stabilization of each carotenoid. It is possible, in theory at least, that some risk factors for ARM, such as family history of ARM and age,

could be associated with a lack of GSTP1, and thus explain our findings.

Alternatively, however, it is possible that our finding that risk for ARM is not associated with the typical significant and positive relationships between MP and serum concentrations of Z, and the relative lack of MP and serum Z seen in association with many of these risk factors, reflects the compromised ability of the retina to stabilize this carotenoid. For example, there is a growing body of evidence that oxidative stress is etiologically important in the pathogenesis of ARM, and it is known that Z acts as an antioxidant within the retinal tissues. It is reasonable to hypothesize, therefore, that a retina at particularly high risk of ARM represents a high oxidative stress environment, and would be associated with excessive depletion of Z, and a consequentially reduced MP optical density and an attenuated relationship, if any, with serum levels of this carotenoid.

Of note, our finding that serum concentrations of Z (adjusted for dietary intake) were significantly reduced in association with three established risk factors for this condition (age, tobacco use, and BMI) in healthy young and middle-aged subjects, is consistent with the finding of Gale et al.[6] that ARM is associated with a relative lack of serum Z. With respect to our finding that heavy cigarette smokers also did not demonstrate a positive and significant relationship between MP optical density and serum levels of L, we offer the same explanation as for Z.

However, the fact that MP represents a composite measure of retinal L, Z, and meso-Z may confound any interpretation of our findings. For example, Z dominates the central foveal region, whereas L is typically dominant in the perifoveal region.[61,63] *meso*-Z [(3*R*,3′*S*)-β,β-carotene-3,3′diol], a carotenoid not found in the normal human diet, is observed to reach its maximum concentration at the foveola, at the same point where the L to Z ratio reaches a minimum.[61,64,65] Of interest, it has been shown that the L proportion of MP decreases with increasing proportion of meso-Z. It has been suggested that L undergoes a chemical oxidation in the central retina (double bond isomerization), and is oxidized to meso-Z.[5,64] Such a conversion, within the retina, may cause changes in the anatomic distribution of MP. For example, a person with large amounts of serum L might be expected to have high peak MP (as this is where meso-Z is found in the retina). Therefore, the relationship between MP and each of its two constituent carotenoids within serum will be influenced by the amount of L that is converted to *meso*-Z at the precise retinal location where MP optical density is being measured. However, a detailed in vitro study would be needed to explore such an effect. Of note, there is no evidence to suggest that metabolic transformations involving L and Z (L to *meso*-Z) occur in serum.[64] Consistent with this view, a recent study in which rhesus monkeys, reared on carotenoid-free diets, were fed L or Z reported an absence of serum Z in the L-fed group and only trace amounts of 3′didehydrolutein (a variant of L) in the Z-fed group, suggesting that such interconversion between L and Z in the serum is negligible.[66] In any case, all in vivo techniques that measure MP do so without distinguishing between retinal L, Z, or *meso*-Z (because the optical properties are identical for these three compounds).

In conclusion, there is no demonstrable relationship between serum levels of Z and MP optical density in heavy cigarette smokers, subjects with a family history of ARM, older subjects and subjects with a BMI > 27, and no demonstrable relationship between serum L and MP optical density in heavy smokers. The finding that risk for ARM is not associated with the typical positive and significant relationship between serum Z and MP optical density in subjects without such risk factors, and decades before the onset of disease, would be consistent with compromised retinal capture and/or stabilization of this carotenoid in maculae predisposed to ARM.

References

1. Klaver CCW, Wolfs RCW, Vingerling JR, Hofman A, De Jong PTVM. Age-specific prevalence and causes of blindness and visual impairment in an older population: The Rotterdam Study. *Arch Ophthalmol*. 1998;116:653–658.
2. Klein R, Wang Q, Klein BEK, Moss SE, Meuer SM. The relationship of age-related maculopathy, cataract, and glaucoma to visual-acuity. *Invest Ophthalmol Vis Sci*. 1995;36:182–191.
3. Beatty S, Koh HH, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000;45:115–134.
4. Snodderly DM, Auran JD, Delori FC. The Macular Pigment 2; spatial-distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674–685.
5. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802–1811.
6. Gale CR, Hall NF, Phillips DIW, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2003;44:2461–2465.
7. Mares-Perlman JA, Fisher AI, Klein R, Block G, Millen AE, Wright JD. 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.
8. Curran-Celantano J, Hammond BR, Ciulla T, Cooper DA, Pratt LM, Danis RB. Relationship between dietary intake, serum concentrations and retinal concentrations of lutein and zeaxanthin in adults in a midwest population. *J Clin Nutr*. 2001;74:796–802.
9. Hammond BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB et al. Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci*. 1997;38:1795–1801.
10. Bone RA, Landrum JT, Dixon Z, Chen Y, Lerena CM. Lutein and zeaxanthin in the eyes, serum and diet of human subjects. *Exp Eye Res*. 2000;71:239–245.
11. Rock C, Thornquist MD, Neuhouser ML, et al. Diet and lifestyle correlates of lutein in the blood and diet. *J Nutr*. 2004;132:525s–530s.
12. Nolan J, O'Donovan O, Kavanagh K, et al. Macular pigment and percentage of body fat. *Invest Ophthalmol Vis Sci*. 2004;45:3940–3950.
13. Burke JD, Curran-Celentano J, Wenzel AJ. Diet and serum carotenoid concentrations affect macular pigment optical density in adults 45 years and older. *J Nutr*. 2005;135:1208–1214.
14. 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.
15. Hammond BR, CurranCelentano 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.
16. Bodner CH, Soutar A, New SA, et al. Validation of a food frequency questionnaire for use in a Scottish population: correlation of antioxidant vitamin intakes with biochemical measures. *J Hum Nutr Diet*. 1998;11:373–380.
17. Masson LF, McNeill G, Tomany JO, et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr*. 2003;6:313–321.
18. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. *McCance and Widdowson's The Composition of Foods*. (5th revised extended ed). Cambridge and London: Royal Society of Chemistry; 1991.
19. O'Neill ME, Carroll Y, Corridan B, et al. A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *Br J Nutr*. 2001;85:499–507.
20. United States Department of Agriculture. USDA-NCC Carotenoid Database for U.S. Foods. 1998.
21. Foods Standards Agency. *McCance and Widdowson's The Composition of Foods*. (6th Summary Ed). Cambridge, UK. Royal Society of Chemistry; 2002.

22. Rand WM, Windham CT, Wyse BW, Young VR. *Food Composition Data: A User's Perspective.* Toyko: United Nations University Press; 1987.
23. Rand WM, Windham CT, Wyse BW, Young VR. *Compiling Data for Food Composition Databases.* New York: United Nations University Press; 1991.
24. Willett WC. Issues in analysis and presentation of dietary data. *Nutritional Epidemiology.* (2nd ed.) New York: Oxford University Press; 2006;321–345.
25. Hammond BR, Wooten BR, Smollon B. Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optom Vision Sci.* 2005;82:387–404.
26. Mellerio J, Ahmadi-Lari S, van Kuijk FJGM, Pauleikhoff D, Bird AC, Marshall J. A portable instrument for measuring macular pigment with central fixation. *Curr Eye Res*. 2002;25:37–47.
27. Hammond BR, Wooten BR, Snodderly DM. Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. *Vision Res*. 1996;36:3003–3009.
28. Mares-Perlman JA, Klein R, Klein BEK, et al. Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch Ophthalmol*. 1996;114:991–997.
29. Seddon JM, Ajani UA, Sperduto RD. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA*. 1994;272:1413–1420.
30. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BEK, 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.
31. Smith W, Mitchell P, Webb K, Leeder SR. Dietary antioxidants and age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmology*. 1999;106:761–767.
32. Flood V, Wang JJ, Manzi F, Webb K, Mitchell P. Dietary antioxidant intake and incidence of early age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmology*. 2002;109:2272–2278.
33. Cho EY, Seddon JM, Rosner B, Willett WC, Hankinson SE. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch Ophthalmol*. 2004;122:883–892.
34. Goldberg J, Flowerdrew G, Smith E. Factors associated with age-related macular degeneration: analysis of data from NHANES I. *Am J Epidemiol*. 1998;128:700–711.
35. Snellen ELM, Verbeek ALM, van den Hoogen GWP, Cruysberg JRM, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand*. 2002;80:368–371.
36. Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol*. 1998;82:907–910.
37. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys*. 2001;385:28–40.
38. Michaud DS, Giovannucci EL, Ascherio A, et al. Associations of plasma carotenoid concentrations and dietary intake of specific carotenoids in samples of two prospective cohort studies using a new carotenoid database. *Cancer Epidemiol Biomarkers Prev*. 1998;7:283–290.
39. Curran-Celentano J, Hammond BR, Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. *Am J Clin Nutr*. 2001;74:796–802.
40. Carroll YL, Corridan BM, Morrissey PA. Carotenoids in young and elderly healthy humans: dietary intakes, biochemical status and diet-plasma relationships. *Eur J Clin Nutr*. 1999;53:644–653.
41. Hammond BR, Ciulla TA, Snodderly DM. Macular pigment density is reduced in obese subjects. *Invest Ophthalmol Vis Sci*. 2002;43:47–50.
42. Ciulla TA, Curran-Celantano J, Cooper DA, et al. Macular pigment optical density in a midwestern sample. *Ophthalmology*. 2001;108:730–737.
43. AREDS. Risk factors associated with age-related macular degeneration: a case-control study in the age-related eye disease study: age-related eye disease study report number 3. *Ophthalmology.* 2000;107:2224–2232.
44. Yannuzzi LA, Sorenson JA, Sobel RS, et al. Risk-factors for neovascular age-related macular degeneration. *Arch Ophthalmol*. 1992;110:1701–1708.
45. Blumenkranz MS, Russell SR, Robey MG, Kottblumenkranz R, Penneys N. Risk-factors in age-related maculopathy complicated by choroidal neovascularization. *Ophthalmology*. 1986;93:552–558.
46. Sperduto RD. Antioxidant status and neovascular age-related macular degeneration. *Arch Ophthalmol*. 1993;111:104–109.
47. 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.
48. Delcourt C, Cristol JP, Tessier F, Leger CL, Descomps B, Papoz L. Age-related macular degeneration and antioxidant status in the POLA study. *Arch Ophthalmol.* 1999;117:1384–1390.
49. Smith W, Mitchell P, Rochester C. Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. *Am J Ophthalmol*. 1997;124:838–840.
50. Broekmans WMR, Berendschot TTJM, Klopping-Ketelaars IAA, 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.
51. Krinsky NI, Russett MD, Handelman GJ, Snodderly DM. Structural and geometrical-isomers of carotenoids in human plasma. *J Nutr*. 1990;120:1654–1662.
52. Rock C, Swendseid M. Plasma carotenoid levels in anorexia nervosa and obese patients. *Methods Enzymol*. 1993;214:116–123.
53. Brady WE, MaresPerlman JA, Bowen P, StacewiczSapuntzakis M. Human serum carotenoid concentrations are related to physiologic and lifestyle factors. *J Nutr*. 1996;126:129–137.
54. Clevidence BA, Bieri JG. Association of carotenoids with human plasma-lipoproteins. *Methods Enzymol*. 1993;214:33–46.
55. Stuerenburg HJ, Ganzer S, Arlt S, Muller-Thomsen T. The influence of smoking on plasma folate and lipoproteins in Alzheimer disease, mild cognitive impairment and depression. *Neuro Endocrinol Lett*. 2005;26:262–263.
56. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am Soc Clin Nutr*. 1996;63:559–565.
57. Olmedilla B, Granado F, Blanco I, Rojashidalgo E. Seasonal and sex-related variations in 6 serum carotenoids, retinol, and alpha-tocopherol. *Am J Clin Nutr*. 1994;60:106–110.
58. Neelam K, O'Gorman N, Nolan J, et al. Measurement of macular pigment: Raman spectroscopy versus heterochromatic flicker photometry. *Invest Ophthalmol Vis Sci*. 2005;46:1023–1032.
59. Hammond BR, Fuld K, CurranCelentano J. Macular pigment density in monozygotic twins. *Invest Ophthalmol Vis Sci*. 1995;36:2531–2541.
60. Yemelyanov AY, Katz NB, Bernstein PS. Ligand-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retina. *Exp Eye Res*. 2001;72:381–392.
61. Snodderly DM, Handelman GJ, Adler AJ. Distribution of individual macular pigment carotenoids in central retina of macaque and squirrel-monkeys. *Invest Ophthalmol Vis Sci*. 1991;32:268–279.
62. Bhosale P, Larson AJ, Frederick JM, Southwick K, Thulin CD, Bernstein PS. Identification and characterization of a pi isoform of glutathione S-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye. *J Biol Chem*. 2004;279:49447–49454.
63. 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.
64. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci*. 1993;34:2033–2040.
65. 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.
66. Neuringer M, Sandstrom MM, Johnson EJ, Snodderly DM. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2004;45:3234–3243.