Macular Pigment and Percentage of Body Fat

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Purpose. To investigate the relationship between percentage of body fat and macular pigment (MP) optical density.

Methods. One hundred healthy subjects of ages between 22 and 60 years volunteered to participate in this study. MP optical density was measured psychophysically, serum lutein and zeaxanthin were quantified by HPLC, and dietary intake of lutein and zeaxanthin was assessed using a validated food frequency questionnaire. Body fat was measured by dual energy x-ray absorptiometry (DEXA); body mass index (BMI) was also calculated for each subject. Clinical and personal details were recorded, with particular attention directed toward putative risk factors for AMD.

Results. There was a significant inverse relationship between the percentage of body fat and MP optical density in males ($r = -0.392$, $P < 0.01$), and after correcting for age and dietary lutein and zeaxanthin, this inverse relationship remained significant ($r = -0.290$, $P < 0.05$). The relationship between MP optical density and percentage of body fat in females was inverse, but not significant ($r = -0.197$, $P = 0.149$). A significant and inverse relationship between serum zeaxanthin and percentage of body fat was observed for females only ($r = -0.354$, $P < 0.01$). Dietary intake of fat was inversely related to serum lutein and zeaxanthin, and significantly so for lutein ($r = -0.256$, $P < 0.05$). However, dietary fat was unrelated to MP optical density ($r = 0.041$, $P = 0.688$).

Conclusions. A relative lack of MP is associated with adiposity in men, and may underlie the association between body fat and risk for AMD progression in males. Further, the processes governing accumulation and/or stabilization of lutein and zeaxanthin in fat tissue appear to differ for males and females. (*Invest Ophthalmol Vis Sci.* 2004;45:3940–3950) DOI: 10.1167/iovs.04-0273

Age-related macular degeneration (AMD), which damages central vision, is the late stage of age-related maculopathy (ARM), and is the leading cause of blindness in elderly people in the Western World.[1] Under physiological conditions, lutein and zeaxanthin accumulate at the macula to the exclusion of all other carotenoids, and are collectively known as macular pigment (MP). The function of MP remains uncertain, but it is believed to reduce chromatic aberration as a result of its absorption spectrum which peaks at 460 nm (blue light).[2] These absorptive characteristics, alone or in combination with the capacity of the retinal carotenoids to quench reactive oxygen intermediates (ROIs), allow the MP to protect the retina from oxidative damage.

Although the pathogenesis of AMD remains unclear, there is a growing body of evidence implicating oxidative stress, which refers to tissue damage arising from interaction between the constituent molecules of a tissue and ROIs. The retina is an ideal site for the generation of ROIs because of its high oxygen consumption, its wealth of chromophores, and its exposure to short wavelength visible light.[3] The threshold for retinal injury induced by visible light, known as photochemical damage, is lowest for blue light.[4] It would seem, therefore, that the optical, antioxidant, and anatomic properties of MP render it suitable to protect against AMD. The dietary origin of MP renders this hypothesis all the more provocative.

Body fat is of particular interest, because adipose tissue is a major storage organ for carotenoids.[5,6] Therefore, variation in body fat may influence carotenoid levels found in serum and other tissues which accumulate carotenoids, such as the retina. Consistent with this, an inverse relationship exists between MP optical density and body mass index (BMI), and percentage of body fat as assessed by bioelectric impedance (BIA).[7]

Obesity, defined as an excess of body fat, increases the risk of progression to advanced AMD, and therefore may be an independent risk factor for ARM.[8] Of note, several large population-based studies have found an association between incidence and/or prevalence of AMD and BMI.[9–13] BMI lends itself as a measure of obesity in epidemiologic studies, largely because it is quick and easy to record. However, the accuracy of BMI (a simple weight to height ratio) as a measure of fat mass, and consequently as a tool for investigating its relationship with carotenoid levels in the blood and retina, remains controversial.[14–17]

The present study was designed to investigate the relationship between MP optical density and percentage of body fat as measured by dual energy x-ray absorptiometry (DEXA), which is the most available direct assessment of fat mass. The relationship between adiposity and serum concentrations of lutein and zeaxanthin was also evaluated.

Methods

Subjects

One hundred healthy subjects volunteered to participate in this study, which was approved by the Research Ethics Committee of Waterford Regional Hospital and the Ethics Committee of the Waterford Institute of Technology. Informed consent was obtained from each volunteer, and the experimental procedures adhered to the tenets of the Declaration of Helsinki.

Subjects were recruited to this single-visit study at the Waterford Institute of Technology by a self-selected sample population who volunteered as a result of posters, newsletters, and word of mouth in the local community. Subjects had to be white between the ages of 20 and 60 years. Volunteers with ocular pathology were excluded.

The following details were recorded for each volunteer: demographic data; general health status, with particular attention directed

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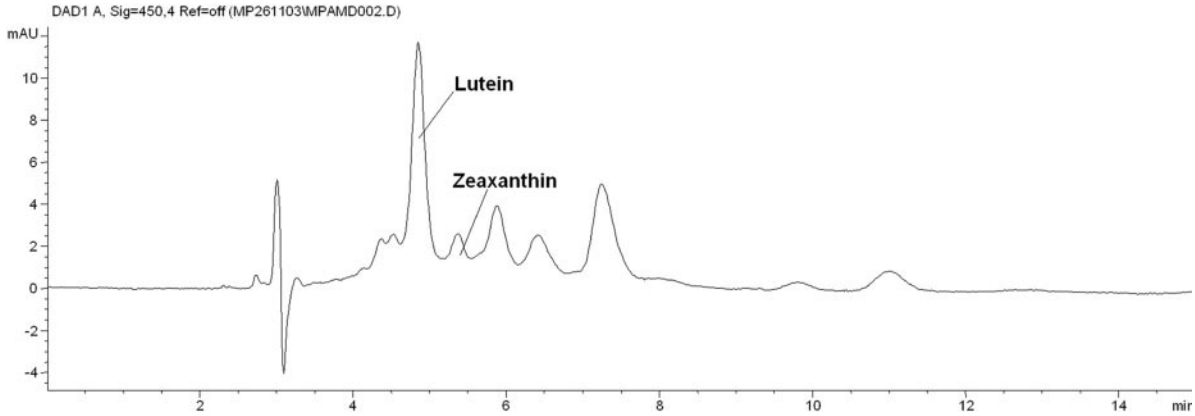


FIGURE 1. HPLC profile showing lutein and zeaxanthin separated using a 5-micron analytical/preparative 4.6 × 250 mm 201TP speciality reversed-phase column.

toward cardiovascular disease or respiratory disease; refractive status; use of medications or dietary supplements; dietary habits detailed in a food frequency questionnaire. Examination included recordings of the following: visual acuity (Snellen and Logmar); macular pigment optical density; iris color (light: blue, green, gray; dark: brown, hazel); retinal photography (digital fundus camera); smoking habits (never, current, or past); and body composition (BMI and DEXA).

Food Frequency Questionnaire

Dietary intake was assessed by a self-administered, semiquantitative food frequency questionnaire (FFQ) developed by the Scottish Collaborative Group, based on questions used in the Scottish Heart Health Study,[18] and previously validated against weighed food records and biomarkers.[19–22]

The questionnaire consisted of 166 specific foods or food types grouped into 19 food groups. A portion or measure for each food was specified, and subjects were asked to record how many measures per day and how many days per week they consumed the food, ranging from "rarely or never" to "7 days per week." A 'measure' was designed to be a small portion so that a single standard portion of a food would often be two measures. Subjects were asked to recall their frequency of consumption over the preceding 2 to 3 months. The questionnaire included an example of how to fill in the questionnaire and a color photograph depicting examples of food measures. It was completed by the volunteer in the presence of the primary investigator (JN), and took between 20 and 30 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 by the Department of Environmental & Occupational Medicine, University of Aberdeen, Aberdeen, Scotland, with software (Relational Database Management System, version 7; Oracle) that incorporated food composition data based on McCance & Widdowson's *The Composition of Foods*.[23] Dietary intake of lutein and zeaxanthin was calculated using food composition data sources from the United Kingdom, Europe, and the United States.[24,25] Standard principles or criteria for the matching of food items and standardized recipes or manufacturer's ingredient information were applied where necessary.[26–28]

Serum Carotenoid 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, and then aliquoted into three light-sensitive microcentrifuge tubes and stored at −70°C until time of analysis. Serum lutein and zeaxanthin were determined by a reversed-phase HPLC system (HP 1090 LC; Agilent, Dublin, Ireland) with photodiode array detection at 295, 325, and 450 nm, and software by Agilent Chem Station. A 5-micron analytical/preparative 4.6 × 250 mm 201TP speciality reversed-phase column (Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase of 97% methanol and 3% tetrahydrofuran was degassed using an in-line degasser. The flow rate was 1 mL/min. Lutein and zeaxanthin standards were provided by Hoffmann-La Roche (Basel, Switzerland).

The extraction procedure was as follows: 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 resulting 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 using a sample concentrator (Techne Sample Concentrator; Davison & Hardy Ltd.). These dried samples were reconstituted in methanol (200 μL), and 150 μL was injected for HPLC analysis. A typical HPLC profile showing lutein and zeaxanthin is illustrated in Figure 1. The assay has been validated against the National Institute of Standards and Technology (NIST) Standard Reference Material 968c for Fat-Soluble Vitamins, Carotenoids and Cholesterol in Human Serum.

Measurement of Macular Pigment

Heterochromatic Flicker Photometry (HFP). MP absorbs blue light and is optically undetectable at 6°–8° eccentricity.[29] HFP is based on the premise of matching two lights (one blue and one green) for equal brightness when their image is foveal, and the test is then repeated when the image is parafoveal. If the green light, for example, remains a constant luminance and the blue light is varied in intensity to match it, the ratio of the amount of blue light required to achieve the endpoint of matching luminance, or minimum flicker, for foveal and parafoveal readings, is a measure of the amount of pigment present, and the logarithm of this ratio represents the optical density of MP.

The Maculometer (John Mellerio, School of Biosciences, University of Westminster, London, UK) makes the matching uncomplicated by flickering the blue and green lights on and off in counter phase, and the subject adjusts the blue light luminance until there is minimum flicker (matching luminance). The subject never observes a state of no flicker because the flickering lights are of different colors. With a minimum of practice, a subject can usually achieve matching luminance without difficulty.

Apparatus. The Maculometer is a small, portable instrument which uses light emitting diodes (LEDs) as light sources.[30] LEDs are good light sources for portable instruments because they are small, inexpensive, are easily driven from simple power supplies, and emit

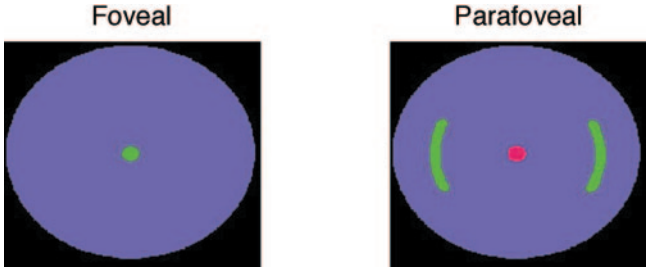


FIGURE 2. Foveal and parafoveal targets observed on the maculometer.

near-monochromatic light. The Maculometer provides central fixation for both the foveal and parafoveal readings because the central test field (foveal) is surrounded by two arcuate test fields (parafoveal), as in Figure 2. The image is viewed at a distance of 330 mm and subtends a diameter of 1° at the eye. Where there is no optically detectable MP, the test field consists of two arcs, representing an annulus concentric with the fovea. This annulus has a diameter of 10°, and a width of 1°. To match luminance in the parafovea, the foveal field is switched from the flickering blue and green light to dim red to provide a fixation target. Thus, the subject always fixates the central 1-degree field, first for the foveal match when it flickers blue/green and the arcs are extinguished, and second on a red fixation target when the arcs are flickering blue/green.

Frequency of Flicker. Frequency of flicker is important to ensure that the matches were made without either rods and/or S cones taking part. This is achieved by arranging the frequency of switching from blue to green to be above the critical fusion frequency for rods. In the parafoveal arcuate fields, this frequency is set to 13 Hz, and in the foveal field 18 Hz. The frequency is higher in the foveal field so that it is also above the critical frequency of the S cones, should any such cones be unadapted by the blue background. The blue and green LEDs are driven with 50% mark-space ratio square wave current pulses in exact counter phase.

Procedure. The Maculometer was set up on a tabletop in a well-lit office, at an angle of approximately 35°. The principles of making a minimum-flicker match were explained to each subject before recording results. Subjects were allowed to make two or three trial minimum-flicker matches before recording of the measurements

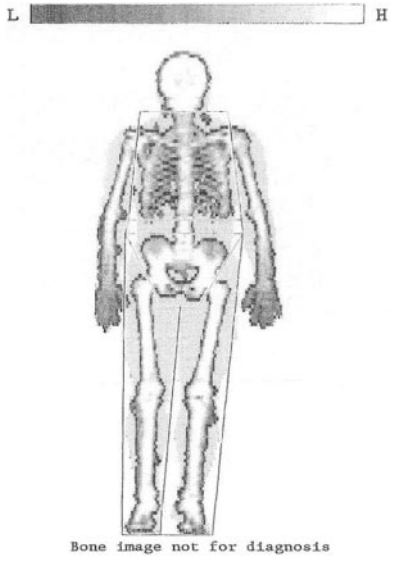


FIGURE 3. DEXA scan obtained for a subject with a percentage body fat of 12%.

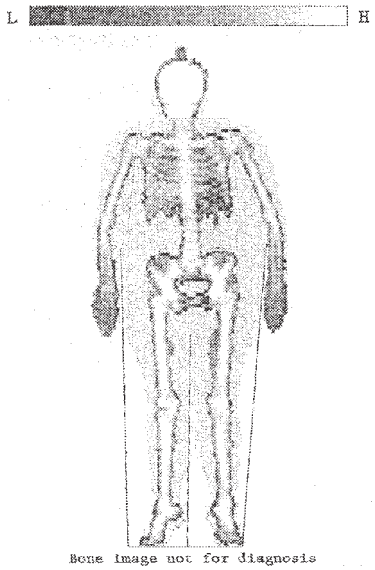


FIGURE 4. DEXA scan obtained for a subject with a percentage body fat of 51%.

commenced, and then were encouraged to make the matches quickly. Perfectionist adjustment of the control was actively discouraged, because a point of no flicker cannot be achieved. The subject's perception of the end-point was identified by adjusting a dial which controls the luminance of the blue-light LED, and, when satisfied, the subject pressed the sample and hold button. After each match was recorded, the investigator set the luminance control to a new arbitrary position so that the subject could not learn how far to adjust the dial to obtain a match. Foveal readings were obtained, followed by parafoveal readings, for each eye.

Reproducibility and Test–Retest Variability. Intersessional variability of MP optical density values obtained psychophysically was assessed by comparing measurements taken on two occasions separated by at least 90 minutes in 100 consecutive volunteers before commencement of this study. Agreement between readings recorded on the two separate occasions was represented by a mean difference of -0.01 ± 0.08, and 95% limits agreement were -0.01 ± 0.16. The reproducibility of the psychophysical measurements was expressed in terms of the coefficient of variation and coefficient of repeatability using six readings of MP optical density recorded during a single session in 100 consecutive volunteers. The mean coefficient of variation was $16.14 \pm 18.48\%$, and the mean coefficient of repeatability was 0.025 ± 0.011.

Body Composition Evaluation. Body mass index (BMI) and dual energy x-ray absorptiometry (DEXA) were used for each subject.

Dual Energy X-Ray Absorptiometry (DEXA). Percentage of body fat was determined for each subject using this gold standard method from a low radiation DEXA scan (Norland XR-46, Norland Medical Systems, Fort Atkinson, WI). DEXA technology, originally developed to measure bone mineral density, can be used for direct assessment of fat mass. The attenuation by tissues of two x-ray intensities was determined and compared to known values for fat and lean tissue.

Subjects were placed lying supine on the DEXA bed, ensuring that they were within the scanning limits. A laser diode (red 670 nm, <0.2 mW) was used to mark a point 1 cm above the center of the subject's head. The laser dot was then positioned at a point on the abdomen adjacent to the spine and midway between the lowest rib and the iliac crest. The position was marked in the area of maximum soft tissue and no bone. The scan was started and the subject was scanned from head to toe. The scan took between 4 and 5 minutes, depending on the height of the person. Figures 3 and 4 illustrate body scans obtained

TABLE 1. Demographic and Visual Data of 100 Subjects

Characteristic	*n* (%)
Age (y)	
22–35	34
36–48	30
49–60	36
Gender	
Male	45
Female	55
Smoking status*	
Current smokers	14
Nonsmokers	86
Family history	
Clinically confirmed family history of ARM	16
No known family history of ARM	84
BMI†	
Desirable weight	53
Overweight	33
Obese	14
Visual acuity better than 6–9	100

* Current smokers smoked at least one cigarette per day. Nonsmokers never smoked or had given up for at least one year.

† Body weight is based upon body mass index (BMI). (BMI = Body mass in kg ÷ height in m^2). Desirable weight represented by a BMI of <24.9, Overweight is between 25 and 29.9, and Obese is >30.

using DEXA from subjects with percentage of body fats of 12% and 51%, respectively.

Obesity was defined as >25% body fat for males, and >32% body fat for females.

Body Mass Index (BMI). The BMI, a weight-to-height ratio, was obtained by recording the person's height (m) and weight (kg). BMI is calculated as kg/m^2.

Statistical Analysis

The statistical software package SPSS, version 11 (SPSS, Chicago, IL), was used for analysis. Coefficient of variation and coefficient of repeatability were calculated to assess reproducibility of HFP measurements within a session, and test–retest variability was assessed by calculating the 95% limits of agreement between readings taken during two separate sessions. Multiple linear regression analysis was used to assess the relationships between a dependent variable and multiple potential independent variables. Pearson correlation coefficients were calculated to investigate the relationship between bivariables and partial correlations when controlling for confounding variables. The significance between group differences was determined by one-way ANOVA or Student's *t* tests, depending on the analysis in question. Significance was set at $P < 0.05$.

RESULTS

The demographic, anthropometric, and visual data of the subjects are summarized in Table 1. The mean age (±SD) of the sample was 42.78 ± 11.67 years, and ranged from 22 to 60 years. Each of the three age tertiles accounted for approximately one-third (30%–36%) of the total.

The relationship between MP optical density and serum and dietary levels of its constituent carotenoids, as well as other dietary indices, are given in Table 2. In brief, it was noted that MP optical density was positively and significantly related to serum levels of lutein (Fig. 5, $r = 0.215$, $P < 0.05$), and zeaxanthin (Fig. 6, $r = 0.214$, $P < 0.05$), and to dietary intake of zeaxanthin ($r = 0.259$, $P < 0.02$). The relationship between the optical density of MP and dietary intake of its constituent carotenoids, and the relationship between dietary and serum levels of lutein and zeaxanthin was not statistically meaningful within any of the subgroups of obese or non-obese males or females, probably reflecting the small size of these subgroups. The relationship between serum lutein and MP optical density was positive for males and females, and significantly so for females where a partial correlation coefficient of 0.28 ($P < 0.05$) was observed after adjusting for dietary lutein, body fat, and age.

Dietary intake of fat was inversely related to serum lutein and zeaxanthin after adjusting for dietary intake of these carotenoids, and significantly so for lutein (Fig. 7, $r = -0.256$, $P < 0.05$). However, dietary fat was unrelated to MP optical density ($r = 0.041$, $P = 0.688$), even when dietary lutein and zeaxanthin were factored into the analysis ($r = -0.0219$, $P = 0.788$).

For any given BMI, females have a higher percentage of body fat than males. Therefore, whenever body fat was introduced into a correlation, the analysis was carried out separately for males and females. Dietary intake, serum concentrations of carotenoids, MP optical density, BMI, and percentage of body fat (DEXA), for males and females are shown in Table 3. Percentage of body fat (measured by DEXA) correlated with the height–weight ratio measurement (BMI) for males ($r = 0.688$, $P < 0.01$), and less so for females ($r = 0.580$, $P < 0.01$).

The association of lifestyle and anthropometric variables (age, smoking habits, family history, BMI, percentage of body fat) on the dietary intake of lutein and zeaxanthin, both alone or in combination, was investigated by multiple linear regression. None of these variables were found to be significantly related to dietary intake of lutein and/or zeaxanthin, ($P > 0.05$, for all), with the exception of age which had a significant inverse relationship with the dietary intake of zeaxanthin for both males and females ($P < 0.05$).

Multiple linear regression was also performed to analyze the relationship between serum lutein (and zeaxanthin), and the following variables: dietary lutein; dietary zeaxanthin; dietary

TABLE 2. Pearson Correlation Matrix Showing Relationship between MP Optical Density and Serum and Dietary Levels of Constituent Carotenoids, and Other Dietary Indices

	Dietary Lutein	Dietary Zeaxanthin	Dietary Fat	Dietary Energy	Serum Lutein	Serum Zeaxanthin	MPOD
Dietary Lutein	1						
Dietary zeaxanthin	0.710**	1					
Dietary fat	0.089	0.209*	1				
Dietary energy	0.139	0.281**	0.899**	1			
Serum lutein	0.265**	0.187	−0.256*	−0.225*	1		
Serum zeaxanthin	0.200*	0.294**	−0.183	−0.168	0.551**	1	
MPOD	0.125	0.259**	0.041	0.048	0.215*	0.214*	1

$n = 100$ (45 males; 55 females).

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

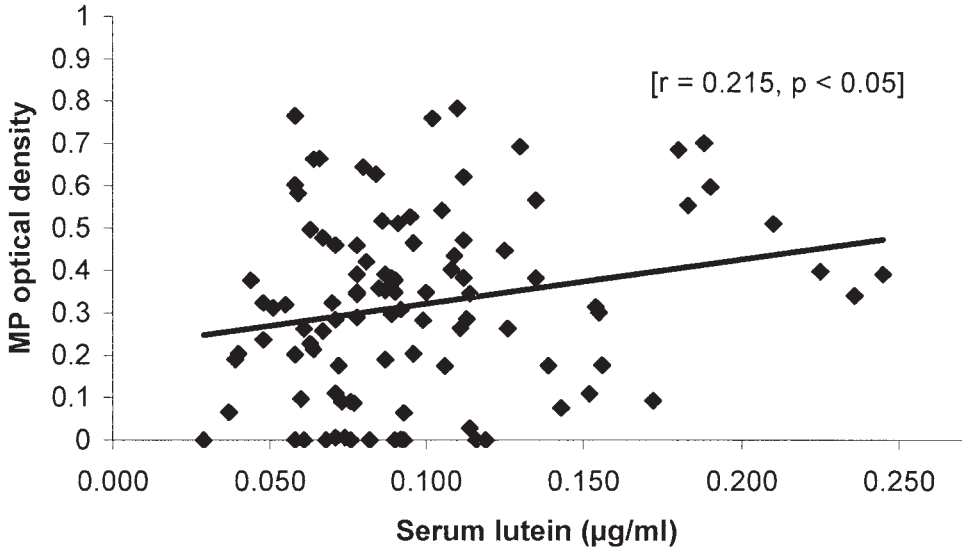


FIGURE 5. Relationship between serum levels of lutein and optical density of macular pigment for 100 healthy subjects. MP optical density, macular pigment optical density; serum lutein, serum levels of lutein (μg/mL).

fat; age; smoking habits; family history of AMD; and body composition (BMI and DEXA entered into separate models). Variables, which were significant predictors of serum lutein and/or zeaxanthin, for males and females, are summarized in Table 4. The relationships between serum lutein (and zeaxanthin), and percentage of body fat (DEXA), were inverse for males and females, but a significant and inverse relationship between serum zeaxanthin and percentage of body fat was observed for females only ($r = -0.354$, $P < 0.01$). Correcting for dietary zeaxanthin did not reduce the strength of this relationship ($r = -0.418$, $P < 0.01$).

Obese females were found to have significantly lower serum concentrations of zeaxanthin (0.023 ± 0.015 μg/mL) when compared with non-obese females (0.039 ± 0.019 μg/mL, $P = 0.028$), and a difference of borderline significance was observed for males (obese males: 0.016 ± 0.01 μg/mL; non-obese males: 0.022 ± 0.013 μg/mL, $P = 0.058$). No statistically demonstrable difference for serum lutein, on the basis of obesity status, was detected for either sex ($P > 0.1$).

Mean (±SD) MP optical density for males with a desirable % body fat (<25) was 0.375 ± 0.205, and this compares with 0.254 ± 0.13 for males with an undesirable percentage of body fat (> 25; $P = 0.06$). The mean (±SD) MP optical density for females with a desirable percentage of body fat (<32) was 0.449 ± 0.25, and this compares with 0.275 ± 0.211 for females with an undesirable percentage of body fat (>32; $P = 0.053$). For males and females, the relationship between serum lutein (and zeaxanthin) and MP optical density was similar for non-obese and obese subjects ($r = -0.04$–0.18, $P > 0.05$, for all).

Body composition (BMI and/or DEXA) and age were the only two variables which demonstrated a significant inverse relationship with MP optical density (multiple linear regression). There was a statistically significant inverse relationship between MP optical density and percentage body fat in males (Fig. 8; $r = -0.392$, $P < 0.01$), even when adjusting for age and dietary intake of lutein and zeaxanthin ($r = -0.290$, $P < 0.05$). The relationship between MP optical density and percentage of body fat in females was inverse, but not significant (Fig. 8; $r = -0.197$, $P = 0.149$).

The inverse relationship between MP optical density and BMI was statistically significant for males ($r = -0.353$, $P < 0.05$), and remained so when age and dietary intake of lutein and zeaxanthin were factored into the analysis (Fig. 9, $r = -0.307$, $P < 0.05$). There was no demonstrable relationship between MP optical density and BMI for females (Fig. 9, $r = -0.019$, $P = 0.892$).

A statistically significant age-related decline in MP optical density was observed for males ($r = -0.446$, $P < 0.01$) and females ($r = -0.300$, $P < 0.05$), and is presented graphically in Figure 10. However, after correction for body fat, the significance of the relationship persisted for males only ($r = -0.378$,

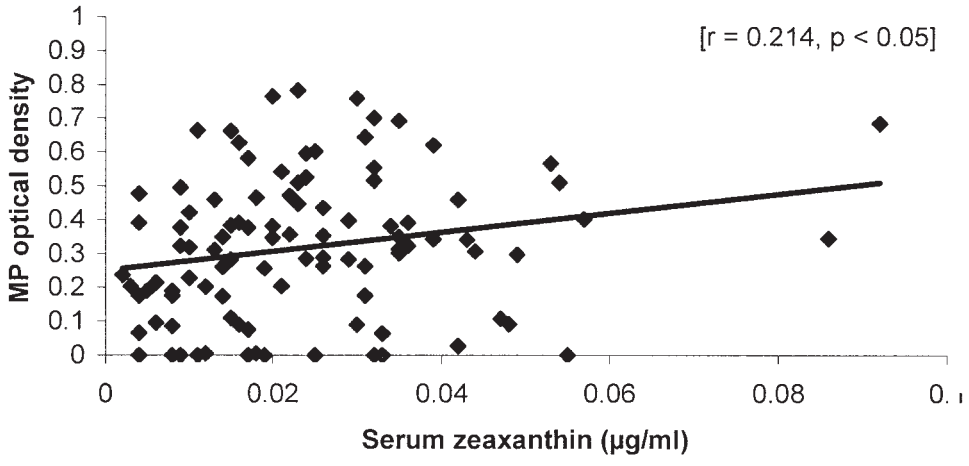


FIGURE 6. Relationship between serum levels of zeaxanthin and optical density of macular pigment for 100 healthy subjects. MP optical density, macular pigment optical density; serum zeaxanthin, serum levels of zeaxanthin (μg/mL).

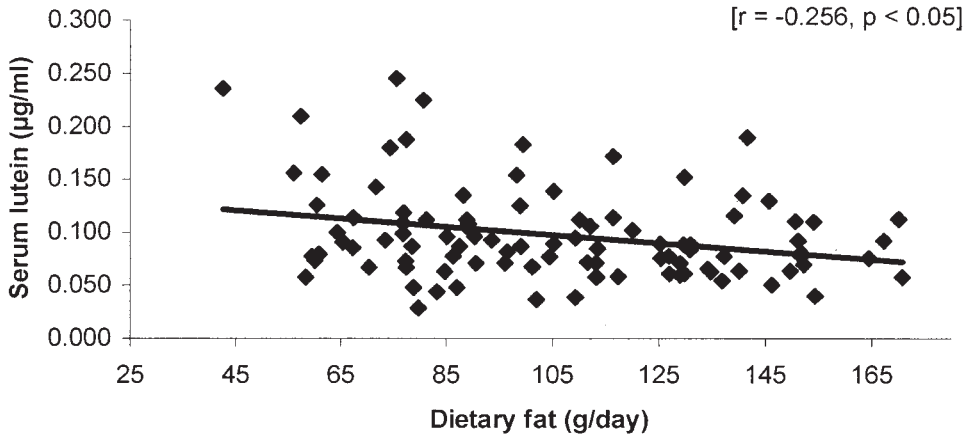


FIGURE 7. Relationship between serum levels of lutein and dietary fat intake. Serum lutein, serum levels of lutein (μg/mL); dietary fat, dietary fat intake (g/day).

$P < 0.05$), and was reduced to borderline significance for females ($r = -0.258$, $P = 0.059$).

DISCUSSION

This study investigated the relationship between percentage of body fat, dietary intake of retinal antioxidants, serum levels of lutein and zeaxanthin, and macular pigment optical density in 100 healthy subjects.

BMI is a method of assessing body composition typically used by investigators studying the relationships between obesity and AMD. However, the moderate level of agreement between BMI and percentage of body fat has prompted criticism regarding the use of BMI as a tool for investigating adiposity for several reasons.[31,32] First, adiposity, rather than excess body mass, is the more important health risk.[33] Second, conclusions regarding the relationship between MP optical density and BMI should be interpreted with caution, as BMI is not a direct assessment of adiposity. DEXA and hydrostatic weighing are regarded as the gold standard techniques in determining body fat. Hydrostatic weighing may be less suitable for studies involving large sample numbers (especially with older subjects), given the need for total submersion in water. Other techniques available, including skinfold thickness and bioelectric impedance, only predict the gold standard measurement (usually that from hydrostatic weighing) using a regression equation. Thus, DEXA represents the most direct assessment of body fat suitable for research of this nature. Of note, this study represents the first investigation of the relationship between MP optical density and adiposity, and between serum lutein (and zeaxanthin) and adiposity, using dual energy x-ray absorptiometry (DEXA).

Only moderate agreement between percentage of body fat (DEXA) and BMI was demonstrated, supporting the view that the latter technique should not be used when a true reflection of adiposity is required. In the absence of DEXA, an expensive technique requiring a considerable level of expertise, other measures of body fat measurement (e.g., bioelectric impedance) should be used in preference to BMI.

Mean MP optical density among our subjects was 0.319, and this is comparable with values ranging from 0.211 to 0.33 for populations of similar age groups.[34–37] Good interocular symmetry of MP optical density was also found, with a mean difference of 0.105; this is comparable with previously published data.[38]

Our results confirm that MP optical density is inversely and significantly related to percentage of body fat in males, even after correcting for age and dietary intake of lutein and zeaxanthin. Further, a significant inverse relationship between MP optical density and BMI in males was demonstrated, which

TABLE 3. Dietary Intake, Serum Concentrations of Carotenoids, MP Optical Density, BMI, and Percentage Body Fat (DEXA), for Males and Females

	Males		Females	
Characteristic	**Value**	**Range**	**Value**	**Range**
Diet				
Lutein (mg/day)	1.20 ± 0.626	0.244–2.506	1.427 ± 0.949	0.228–5.72
Zeaxanthin (mg/day)	0.18 ± 0.090	0.052–0.570	0.215 ± 0.136	0.043–0.810
Lutein + zeaxanthin (mg/day)	1.34 ± 0.670	0.298–2.679	1.605 ± 1.056	0.311–6.198
Serum				
Lutein (μg/mL)	0.093 ± 0.042	0.029–0.245	0.102 ± 0.045	0.039–0.236
Zeaxanthin (μg/mL)	0.021 ± 0.013	0.004–0.053	0.026 ± 0.018	0.002–0.092
MP optical density*				
Right eye	0.329 ± 0.193	0–0.783	0.310 ± 0.228	0–0.766
Left eye	0.362 ± 0.173	0–0.784	0.351 ± 0.213	0–0.849
Body composition				
BMI†	26.54 ± 2.9	22.4–33.83	24.71 ± 3.70	20.06–34.2
% body fat (DEXA)‡	23.11 ± 5.9	4.00–37.00	36.18 ± 6.09	22.00–51.0

Mean ± SD, $n = 45$ males and 55 females.

* MP optical density, optical density of macular pigment.

† BMI, Body mass, Kg ÷ height m^2.

‡ % body fat (DEXA), % body fat measured with duel energy x-ray absorptiometry.

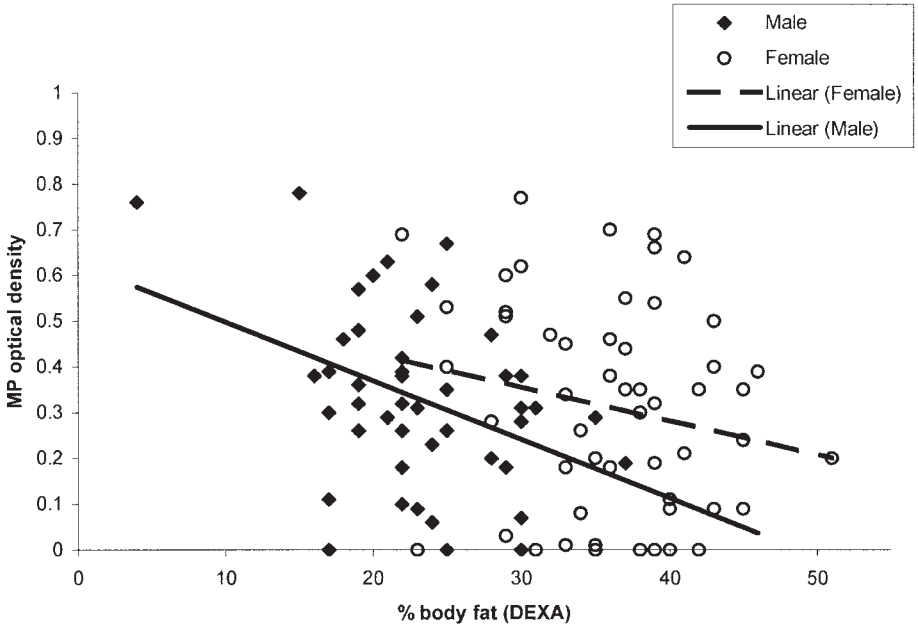
TABLE 4. Association of Lifestyle and Anthropometric Variables on Serum Levels of Lutein and Zeaxanthin for Males and Females

Males		Females	
Dependent Variable	**Significant Explanatory Variable(s)**	**Dependent Variable**	**Significant Explanatory Variable(s)**
Serum lutein	Family history* (P)	Serum lutein	Dietary L** (P)
	Dietary fat* (N)		Dietary fat** (N)
Serum zeaxanthin	Dietary zeaxanthin** (P)		Age** (P)
	Dietary fat* (N)		BMI* (N)
	Smoking habits** (N)		DEXA ($P = 0.092$) (N)
		Serum zeaxanthin	Dietary fat* (N)
			Age* (N)
			BMI* (N)
			DEXA** (N)

(P), positive predictor; (N), negative predictor.
* $P < 0.05$.
** $P < 0.01$.

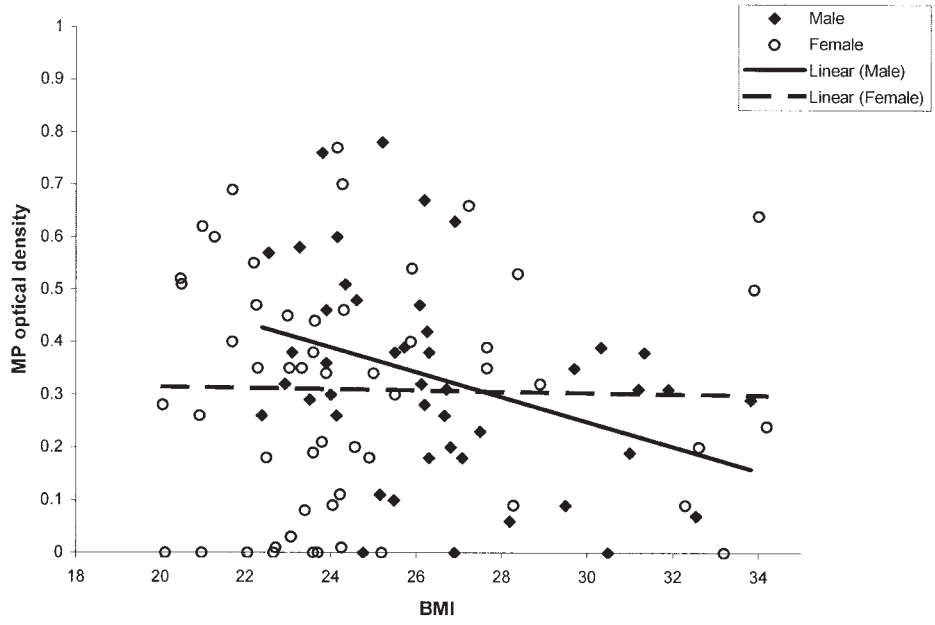
again was maintained after correcting for age and for dietary intake of lutein and zeaxanthin. However, the relationship between BMI and MP optical density was lower than that observed for percentage body fat. The relationship between MP optical density and percentage of body fat in females was inverse, but not significant. There was no demonstrable relationship between MP optical density and BMI in females.

The inverse relationship between percentage of body fat and MP optical density that we observed has also been reported by previous investigators.[7] Several possible explanations account for this finding. First, it has been hypothesized that adipose tissue and retina compete for uptake of lutein and/or zeaxanthin, a hypothesis consistent with the preferential uptake of lutein by fat tissue when compared with the retina,[39] and also in agreement with work carried out by Johnson et al. [40] who demonstrated that changes in adipose tissue lutein concentration were inversely related to changes in MP optical density in women after dietary modification. In other words, these investigators suggested that adipose tissue acts as a sink and a reservoir for lutein. However, the miniscule size of the retina when compared with total body fat, irrespective of obesity status, questions the plausibility of such a hypothesis. If body fat were in direct competition with the retina for absorbed lutein and/or zeaxanthin, a weaker relationship would be expected between dietary intake of these carotenoids and MP optical density, and between dietary intake of these carotenoids and serum levels of these carotenoids, in obese subjects when compared with non-obese subjects. Also expected would be a relative lack of serum lutein and/or zeaxanthin in obese versus non-obese subjects if fat stores were preferentially accumulating these carotenoids. However, the relationship between MP optical density and serum concentrations of its constituent carotenoids, in the context of body fat acting as a sink and competitor for absorbed lutein and



Males, [r = -0.392, p < 0.01]
Females, [r = -0.197, p = 0.149]

FIGURE 8. Relationship between macular pigment optical density and percentage of body fat (DEXA) in 45 males and 55 females. MP optical density, macular pigment optical density; % body fat (DEXA), percentage of body fat measured with DEXA.



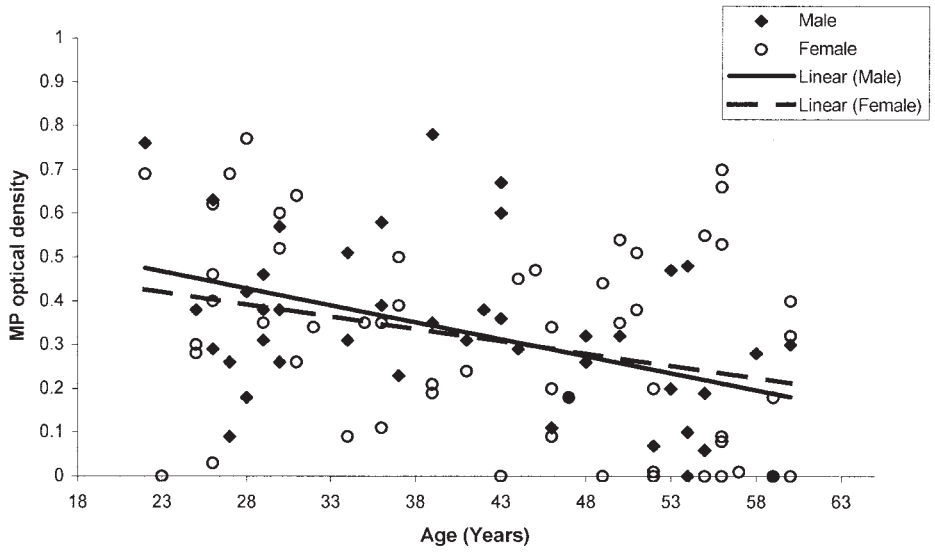
Males, [r = -0.353, p < 0.05]
Females, [r = -0.019, p = 0.892]

FIGURE 9. Relationship between macular pigment optical density and BMI in 45 males and 55 females. MP optical density, macular pigment optical density; BMI, body mass index (body mass, Kg ÷ height, m^2).

zeaxanthin, would be difficult to interpret as both the serum and the macula would be deprived of these compounds.

The relative lack of serum zeaxanthin that we observed for obese females when compared with non-obese females, therefore, supports the hypothesis that adipose tissue acts as a sink for this carotenoid. However, our small sample size did not allow a meaningful comment on the relationships between dietary intake of lutein and zeaxanthin and serum concentrations of these carotenoids, or on the relationship between MP optical density and dietary intake of its constituent carotenoids, in relation to obesity status for each sex.

Johnson et al.[40] and Broekmans et al.[35] have shown that adipose tissue concentrations of lutein are significantly and positively related to MP optical density in men only. Indeed, Johnson et al.[40] also demonstrated a significant and inverse relationship between adipose tissue concentration of lutein and MP optical density in females. The findings of these investigators are consistent with our observation that MP optical density is inversely and significantly related to body fat for males only. For example, one would expect excess body fat in a male, in the context of consistent dietary intake of lutein and zeaxanthin, to result in dilution of the concentration of these carotenoids in adipose tissue and a parallel drop in MP. Whereas, for females, increased body fat will also be associated with reduced fat concentrations of lutein and/or zeaxanthin, but this reduction will not be associated with a parallel decrease in retinal lutein and/or zeaxanthin, since there is no demonstrable relationship between MP optical density and body fat in females. Indeed, our observations are also entirely consistent with an in-depth study[39] of zeaxanthin concentra-



Males, [r = -0.446, p < 0.01]
Females, [r = -0.300, p < 0.05]

FIGURE 10. Relationship between MP optical density and age in 45 males and 55 females. Statistically significant age-related decline in MP optical density is observed. MP optical density, macular pigment optical density; age, age in years.

tions of retina, liver, fat, and serum after supplemental zeaxanthin in carotenoid-deficient quail. In that study, retinal lutein was significantly and inversely related to fat concentration of this carotenoid for females, whereas zeaxanthin concentration of retina was significantly and positively related to fat concentration of this carotenoid in males only. Also, zeaxanthin concentrations of fat increased by factors of 6.5 and 1.5 in females and males after zeaxanthin supplementation, respectively. It appears, therefore, that accumulation and/or stabilization of the macular carotenoids in fat tissue differs for males and females.

However, it is also possible that a relative lack of MP in obese subjects simply reflects a poor diet among those persons, as it has been demonstrated that obesity is associated with reduced dietary intake of the carotenoids which comprise the MP.[7,8] Indeed, we have demonstrated such a significant and inverse relationship between percentage of body fat and dietary intake of lutein and zeaxanthin (mg/day), but the inverse relationship between percentage body fat and MP optical density persisted after adjusting for dietary intake of lutein and zeaxanthin and for dietary intake of fat ($r = -0.25$, $P = 0.012$). In other words, the significant and inverse relationship between MP optical density and adiposity that we observed is not attributable to differences in dietary intake of lutein and/or zeaxanthin. Therefore, our results are consistent with the hypothesis that body fat competes with the retina for uptake of lutein and/or zeaxanthin, but for males only. Whether a larger sample size would detect a similar relationship for females requires further study.

Other possible mechanisms whereby adiposity is related to MP optical density require discussion. For example, high-density lipoproteins (HDL) are the primary carriers of lutein and zeaxanthin, whereas low-density lipoproteins (LDL) transport hydrocarbon carotenoids (e.g., lycopene, β-carotene).[41] Indeed, some investigators[42] have suggested that low particle contents of lutein and zeaxanthin in LDL may underlie reduced tissue targeting of antioxidants in subjects with a dense LDL phenotype. Also, the preferential uptake of HDL by the retina may explain the selective uptake of lutein and zeaxanthin to the exclusion of all other carotenoids.[39] Interestingly, a recent study[43] has shown a 50% prevalence of low HDL-cholesterol (HDL-C $\leq$ 0.91 mM) in overweight and obese males, with only a 10.7% prevalence in overweight and obese females. In other words, reduced HDL in obese males (but not females) may impair transport and delivery of the macular carotenoids to the retina in obese males.

Mean serum levels of lutein and zeaxanthin for males were 0.093 $\pm$ 0.042 μg/mL and 0.021 $\pm$ 0.013 μg/mL, respectively, and for females were 0.102 $\pm$ 0.045 μg/mL and 0.026 $\pm$ 0.018 μg/mL, respectively, which are consistent with those obtained by previous investigators for populations of similar age groups.[35,44–46] The relationship between adiposity and serum lutein, and the relationship between adiposity and serum zeaxanthin, was inverse for males and females, but significantly so for females only. Also, obese females had significantly lower serum concentrations of zeaxanthin (but not lutein) than non-obese females, whereas obese males had lower serum concentrations of zeaxanthin (but not lutein) than non-obese males which approached statistical significance. These findings are consistent with the preferential uptake by fat of zeaxanthin over lutein by a factor of 4:1 in quail, and with the enhanced uptake of zeaxanthin by fat in females compared with male quail.[39] Also, a relative lack of serum zeaxanthin in obese subjects is consistent with the hypothesis that fat tissue acts as a sink for absorbed zeaxanthin, and is rendered all the more provocative by the observation by Gale et al.[47]that a relative lack of serum concentrations of this carotenoid is seen in patients with AMD.

Our data also indicated that dietary intake of fat was significantly and inversely related to serum concentrations of lutein and zeaxanthin, but was not related to MP optical density after adjusting for dietary lutein and zeaxanthin. Possible explanations for this finding include reduced dietary intake of these carotenoids associated with a high fat diet, and/or reduced levels of high density lipoproteins (which transport the majority of lutein and zeaxanthin) which are seen in association with a high fat diet.[48,49] Our findings support the latter hypothesis, as there was no demonstrable inverse relationship between dietary lutein and/or zeaxanthin and dietary intake of fat. Indeed, we have shown a significant and positive relationship between dietary intake of fat (g/day), and dietary zeaxanthin (mg/day), with no significant relationship observed for lutein. Of clinical significance, a recently published study[48] has shown that progression of AMD is associated with a high dietary intake of fat. Our findings are consistent with the hypothesis that this increased risk may be attributable to a parallel and relative lack of serum lutein and/or zeaxanthin in these patients.[47]

In a recent study[8] investigating the association between the progression of age-related macular degeneration (AMD) and obesity, it was found that overall and abdominal obesity were independent risk factors for progression to the advanced forms of AMD. The relationship between adiposity and AMD may be causal or chronological, and several mechanisms have been put forward to account for the association.[8–12,48] For example, some investigators[8,48] have hypothesized that inflammation represents an antecedent common to adiposity and AMD. Others[8,9] have postulated that hypertension and cardiovascular disease, which are associated with obesity and AMD, contribute to the genesis of this condition. Furthermore, others[3] have suggested that oxidative stress, which is independently associated with obesity and with AMD, may represent the link between undesirable body weight and age-related macular disease. Our results, however, are consistent with the hypothesis that a relative lack of MP, and its putative protective effect for AMD, may account for the observed increased risk of progression of AMD in obese male subjects. Whether weight loss results in augmentation of MP, with a consequential protective effect for AMD, will require further study. Interestingly, however, elevated serum levels of carotenoids have been reported in patients suffering from anorexia nervosa.[17]

It was not the aim of this study to investigate the relationship between MP optical density and age. Nonetheless, a significant and inverse relationship between MP optical density and age was demonstrated, and this persisted after correction for body fat. However, when analyzed separately for males and females, the inverse relationship between MP optical density and age was reduced to borderline significance for females ($P = 0.059$), but persisted for males. These findings are consistent with two other studies[50,51] using psychophysical techniques to measure MP optical density, but not with some other published reports.[35,36,52] The age-related decline exhibited among our sample accounted for 12% of the decline observed in MP, with a mean MP optical density of 0.234 $\pm$ 0.222 for the 49–60-year-old age group compared with 0.399 $\pm$ 0.202 for the 22–35 year-old-age group. In contrast, investigators[53,54] using Raman spectroscopy to measure macular pigment levels attributed 44% of this decline to age. The age-related decline in MP reported by investigators[55,56] using Raman and other techniques has been the subject of a lively debate in the published literature, and warrants further study.

In conclusion, we report a significant and inverse relationship between percentage of body fat and MP optical density for males only, which is consistent with the known positive relationship between adipose concentrations of lutein and MP optical density seen in men. We have also demonstrated that serum levels of lutein and/or zeaxanthin are inversely related to

body fat, and to dietary intake of fat, for both sexes. Our findings are consistent with the hypothesis that, in men, a relative lack of MP may underlie the increased risk of AMD progression associated with body fat. Furthermore, a relative lack of serum concentrations of lutein and/or zeaxanthin may explain the increased risk of progression of AMD associated with dietary fat. Finally, our results also indicated that the mechanisms governing accumulation and/or stabilization of the macular carotenoids in fat tissue differ for males and females.

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