Assessment of lutein and zeaxanthin status and dietary markers as predictors of the contrast threshold in two age groups of men and women.

Rocío Estévez-Santiago¹; Begoña Olmedilla-Alonso^{1*}; Beatriz Beltrán-de-Miguel²

¹ Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), Madrid, Spain

² Facultad de Farmacia. Universidad Complutense de Madrid, Madrid, Spain

*Corresponding author:

Begoña Olmedilla-Alonso. Department of Metabolism and Nutrition, ICTAN-CSIC.

José Antonio Novais 10, 28040 Madrid, Spain

Tel: (+34) 91 549 2300 Fax: (+34) 91 549 3627

BOlmedilla@ictan.csic.es.

Abbreviations

AMD, age-related macular degeneration; BMI, body mass index; CI, confidence interval; CS, contrast sensitivity; CT, contrast threshold; F+V, fruit and vegetable; GLM, generalized linear models; HDL, high-density lipoprotein cholesterol; HPLC, high performance liquid chromatography; L+Z, lutein plus zeaxanthin; MP, macular pigment; MPOD, macular pigment optical density; LDL, low-density lipoprotein cholesterol; TG, triglycerides.

Abstract

Lutein and zeaxanthin (L+Z) status is associated with the macular pigment (MP). The relationship between MP and visual function is controversial. We hypothesized that, within the framework of nutrition, visual function was related to MP and nutritional and/or dietary factors influencing it. A cross-sectional study was performed in 108 volunteers divided into two age groups (20-35 y; 45-65 y), each 27 women and 27 men, to assess the relationship between macular pigment optical density (MPOD) and contrast threshold (CT), considering the influence of L+Z and, fruit and vegetable (F+V) intake. MPOD, L+Z in serum and dietary intake were determined using heterochromatic flicker photometry, HPLC and 3-day food records, respectively. CT was measured with the CGT-1000 Contrast Glaretester at 6 stimulus sizes, with and without glare. Spearman correlation coefficient and a generalized linear model were used for the statistical study. MPOD and CT were lower in younger individuals ($p < 0.000$) and were correlated only in the older group. CT were higher under glare conditions, at the intermediate and smaller visual angles, with greater differences in the older ($p < 0.003$) than the younger group ($p < 0.014$). In the total sample, CT correlated inversely with MPOD (correlation coefficients and p values ranging from -0.245 to -0.152 and from 0.000 to 0.026, respectively) and directly with F+V intake (correlation coefficients and p values ranging from -0.265 to -0.176 and from 0.000 to 0.010, respectively). As predictors of CT in the total sample, MPOD, F+V (every 100g/d) and sex were identified (β coefficients ranged from -0.01 to -1.86 ; from 0.01 to 0.08 and from 0.01 to 0.40, respectively). CT revealed age-specific nutritional predictors: MPOD and serum lutein in the 45-65-y group, and F+V intake in the 20-35-y group.

Key words: macular pigment; contrast threshold; lutein / zeaxanthin; fruits / vegetables; age groups; cross-sectional study.

1. Introduction

The macula is located roughly in the center of the retina and is responsible for detailed central vision. The yellow coloration of the macula is due to the presence of the macular pigment (MP) in the axons of its photoreceptors [1] which is composed of lutein, zeaxanthin—major carotenoids in the human diet (mainly from fruits and vegetables)—and meso-zeaxanthin, which is believed to be obtained from the dietary lutein in the retina and, in small amounts, from the diet [2]. These carotenoids are found at the macula in higher concentrations than anywhere else in the body. There they can act as antioxidants to protect the eyes from oxidative stresses and as blue light filters [3] [4]. The MP can be measured by a number of techniques, the most widely used noninvasive test being heterochromatic flicker photometry [5]. On the other hand, there is growing evidence suggesting that some food components can attenuate the risk and/or progression of age-related macular degeneration (AMD), a major cause of blindness in the elderly population in the developed world, and lutein and zeaxanthin are among those components [4].

Numerous studies have measured the MP because incrementing its density has been related to improved visual function [4, 6, 7] and low MP levels are considered a modifiable risk factor for AMD. MP optimizes visual performance in non-diseased eyes because of its pre-receptor absorption of blue light and consequential attenuation of the effects of chromatic aberration and the adverse effect of light scatter [8, 10]. The evidence that the MP carotenoids could improve visual performance is supported by different types of studies, such as those assessing the effect of lutein supplementation on visual performance in patients with cataracts [11] or early stage AMD [7, 12] and in controls [13], which reported an improvement in visual acuity and a reduction of glare sensitivity. These functional improvements were

consistent with measured increases in MP density [12], although a relationship between MP and visual acuity was not always found and there were controversial results depending, mainly, on the stimulus conditions used [14-17] and the ways in which visual function was assessed. In this regard, contrast sensitivity (CS), a measure of the ability of the visual system to distinguish objects of dissimilar luminance, is considered to better reflect overall visual performance than does visual acuity [18].

Although most of the increasing number of observational and interventional studies designed to assess the relationship between macular pigment optical density (MPOD) and visual performance show a significant relationship between variation in MPOD and immediate effects on visual function [19], the cause and effect relationship between lutein and zeaxanthin (L+Z) intake and the maintenance of normal vision has not been established. While it is widely accepted that lutein can increase MPOD in most, but not all, healthy subjects, it has not been established that said increase in MP density be related to vision [20,21], or that the consumption of a the combination of L+Z be related to improved vision under bright light conditions [22]. Thus, further studies are needed to examine the relationship between MPOD and visual function in well-characterized groups of individuals and using comparable methodologies.

Taking into account previously cited studies, the hypothesis of the present study is that, within the framework of nutrition, visual function is related to the MP, as well as to the nutritional / dietary factors that influence MP and that that relationship can differ according to the age of the subjects. Thus, the aim of this cross-sectional study was to assess the relationship between MPOD and CS (assessing the contrast threshold [CT]), with and without glare, as a measurement of visual function / performance, in a group of apparently

healthy Spanish subjects, considering the influence of biochemical and dietary variables that correlate with MPOD in these individuals, as previously reported [23]: age, gender, serum concentration of lutein and of L+Z / cholesterol plus TG, and the fruit and vegetable (F+V) intake.

2. Methods and materials

2.1. Participants and experimental design

One hundred eight volunteers, divided into two age groups (20-35 y and 45-65 y) (means $\pm$ SD: 25.6 $\pm$ 3.2 y and 52.4 $\pm$ 5.2 y, respectively), each including 27 women and 27 men, were enrolled in a cross-sectional study over the course of an entire year and underwent blood sampling, assessment of MPOD and CT (with and without glare) and three 24-hour records. The recruitment and selection process, serum lutein and zeaxanthin concentrations and MPOD are described elsewhere [23]. The age groups were established because of their different dietary habits and risk of age-related eye diseases. Briefly, the inclusion criteria were normal cholesterol, body mass index (BMI) under 30 kg/m² and mixed diet. Volunteers were asked to report information on the following exclusion criteria: consumption of dietary supplements, BMI under 20 kg/m², surgery for myopia (within the previous year), cataracts, AMD and any eye diseases / discomfort they may have, use of drugs or phytosterol-enriched beverages/foods to control cholesterol level, regular consumption of n-3 fatty acid-enriched food products and chronic diseases that can affect carotenoid or lipid metabolism (e.g., diabetes, cardiovascular disease).

This study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the

Clinical Research Ethics Committee of Hospital Universitario Puerta de Hierro-Majadahonda of Madrid, Spain (registry no. 257, dated 19 July 2010). Written informed consent was obtained from all participants.

2.2. Macular pigment optical density (MPOD) assessment

MPOD was assessed using an MPS 9000 desktop device (Macular Pigment Screener, Elektron PLC, Cambridge, UK) that applies the principles of heterochromatic flicker photometry. The technique and reliability of this device are described in detail by van der Veen et al. [24]. The test consisted of two stages for central and peripheral viewing, and the participants were required to press a response button as soon as they detected flicker. The subjects started by fixating the central stimulus, a 1-degree central target (flicker rate was initially set at 60 Hz and then gradually reduced at a rate of 6 Hz s⁻¹). The process was repeated for a series of green-blue luminance ratios. The observer then fixated a red 2°-diameter target placed 8° eccentrically and a second set of data were recorded for peripheral viewing [25]. The MPOD was measured in density units (du) and ranged from 0 to 1. The MPOD was measured with spectacle correction when necessary.

2.3. Contrast threshold

Contrast threshold (CT) was measured with the CGT-1000 Contrast Glaretester (Takagi, Japan), which determined the CT by means of an automated strategy, set for 6 sizes of annular stimuli with diameters ranging from 6.3 to 0.7 degrees of visual angle, with and without glare light conditions. There were 12 levels of CT, ranging from 0.01 to 0.45. Each subject was tested monocularly for CT, once with each eye and with spectacle correction when necessary.

The luminance of the background on which the stimulus was presented was 10 cd/m^2 . The test time for each eye was very short, approximately 2 minutes, which avoided tiring the participants. The specifications selected for the presentation of the stimulus were: presentation duration, 0.2 s; presentation interval, 2 s; test luminance with glare, $40,000 \text{ cd/m}^2$; test distance, 350 mm. The device had 8 glare sources arranged around the stimulus that were activated automatically to assess the CT with a simultaneous glare. The test results were automatically printed out on a single graph that showed the sensitivity functions with and without glare.

2.4. Analysis of lutein and zeaxanthin in blood

Lutein and zeaxanthin levels were determined by high-performance liquid chromatography (HPLC) and the procedure and results have been published elsewhere [23]. Briefly, the HPLC system employed consisted of a model 600 pump, a Rheodyne injector and a 2998 photodiode array (PDA) detector (Waters, Milford, MA, USA). We used a Spheri-5 ODS $5 \mu\text{m}$ ($220 \text{ mm} \times 4.6 \text{ mm}$) chromatographic column (Brownlee Labs, Applied Biosystem, Santa Clara, CA, USA) with a guard column (Aquapore ODS type RP-18). The mobile phase was acetonitrile-methanol (85:15; v/v), and was changed to acetonitrile-dichloromethane-methanol (70:20:10; v/v/v) in a linear gradient from min 5 to min 20. Both mobile phases were stabilized with ammonium acetate (0.025 mol/L) added to the methanol. The flow rate was 1.8 mL min, and detection was performed at a wavelength of 450 nm. All chromatograms were processed using Empower 2 software (Waters, Milford, MA, USA). Carotenoid extraction was performed on serum samples using a slight modification of a previously published method [26]. Briefly, 200 μL of serum was added to 200 μL of ethanol, vortexed and

extracted twice with 400 µl of hexane:dichloromethane (5:1) stabilized with 0.1 g/L butylated hydroxytoluene (BHT). Organic phases were pooled, evaporated to dryness under nitrogen atmosphere and reconstituted with 200 µl of a solution of tetrahydrofuran:ethanol (1:2) and injected (5 µl) onto the HPLC system. Standard solutions were prepared from 1 mg of lutein and of zeaxanthin dissolved in 25 mL tetrahydrofuran, with 0.01% BHT in each case. The $E_{1\%}^{1\text{cm}}$ values and wavelengths used were as follows: lutein, 2550 at 445 nm; zeaxanthin, 2540 at 450 nm. Working solutions were obtained from different volumes of the standard solutions dissolved in tetrahydrofuran:ethanol (1:2 v/v). The concentrations of the carotenoids in the curve were: 0.27-1.36 µg mL for lutein ($R^2 = 0.999$) and 0.03-0.15 µg mL for zeaxanthin ($R^2 = 0.999$).

2.5. Dietary intake assessment

Recent dietary intake was evaluated by 3-day food records involving 24-h recalls, one of which coincided with a weekend or holiday, carried out within a period of 7 to 10 days. For the first recall, the participants underwent a face-to-face encounter with a specialized interviewer, normally the same person who, subsequently, performed the other two recalls by telephone. The amounts consumed were estimated in units (fruits), portions or household servings [27]. On the basis of this information, we calculated food intake in grams/day, which served as the basis for the determination of the daily lutein and zeaxanthin intake using a database that included HPLC analytical data on the carotenoid content of foods [28], incorporated into a software application for the calculation of dietary intake of individual carotenoids [29]. The procedures and results have been published in detail elsewhere [23].

2.6. Statistical analyses

Sample size calculation was performed on the basis of a mean value for MPOD of 0.40 du. A sample of 108 subjects ($SD = 0.10$) was found to be necessary to obtain a 10% difference in the MPOD (0.04 du) with 85% power and an alpha error of 0.05.

Data are expressed as the means and standard deviations, medians and 95% confidence intervals (CI). The normal distribution of the data was assessed (Kolmogorov-Smirnov test) and, as lutein and zeaxanthin in serum and diet and CT did not follow a normal distribution, nonparametric tests were used to compare the values of the variables analyzed. The CS is the inverse of the CT. Correlations among CT and MPOD, fruit and vegetable intake and lutein+zeaxanthin / cholesterol + TG in serum were established using Spearman's rho correlation coefficient.

The statistical models used were generalized linear models (GLM, TWEEDIE distribution and $LINK=LOG$), with CT (six levels of stimuli, visual angle degrees) as the dependent variable and with fixed factors (sex, age) and covariates (F+V intake, lutein and L+Z / cholesterol+ TG in serum and MPOD). The variables assessed as potential predictors of the CT response were the biochemical parameters (lutein in serum, L+Z / cholesterol + TG) and sex and age (because they were seen to be predictors of MPOD in these subjects, as well as the F+V intake (because, among the biochemical and dietary variables, it was the variable that showed the highest coefficient correlation [$p=0.350$] with MPOD), as previously reported [23] and MPOD. Interaction was observed for CT (with and without glare), which was influenced by age and sex in the group of older subjects.

All reported *P*-values are based on a two-sided test and compared to a significance level of 5%. The SPSS v.23 (SPSS Inc., Chicago, IL, USA) software package was used for all statistical calculations.

3. Results

The CT data (means $\pm$ SD, medians and 95% CI) for each of 6 different degrees of visual angle, with and without glare, are shown in table 1. The lower the CT, the higher the CS level at which a subject could detect each spatial frequency. The CT values were higher with glare than without glare at the intermediate and smaller visual angles (2.5, 1.6, 1.0, 0.7 degrees) and the differences were greater in the older group ($p < 0.003$) than in the younger group ($p < 0.014$). No significant differences were found at the large stimulus sizes (6.3 and 4.0 degrees), with or without glare, within each age group. The differences in CT, with and without glare, between the age groups were significant at every stimulus size, with higher CT in the older *vs* younger group ($p < 0.000$). The CT values for each stimulus sizes, with and without glare, were highly correlated, the correlation coefficients being higher in the older group (between 0.635 and 0.904; $p > 0.000$) than in the younger group (between 0.251 and 0.696; $p < 0.001$). There was an interaction between sex and CT in the older group.

Table 2 shows the MPOD of the volunteers, together with data for dietary variables (F+V intake) and biochemical variables (lutein and L+Z / cholesterol + TG in serum), because of their correlations with the MPOD [23]. Fruit and vegetable intake showed a correlation with the biochemical marker in both age groups but, with the MPOD, only in the older group. The significant correlations of those variables with the CT at the six visual angles are shown in table 3. The CT exhibited a significant inverse correlation with MPOD at every visual angle,

with and without glare, in the older but not in the younger group. The F+V intake was also correlated (in this case, directly) with the CT at any visual angle, except 6.3 degrees without glare, in the total sample, but not when age groups were considered. Regarding the biochemical parameters, only one significant correlation was found between the lutein + zeaxanthin / cholesterol + TG in serum and the CT at 6.3 and 1.0 degrees (without glare), in the younger and older group, respectively. In the older group, three were correlations between CT (4.0, 2.5 and 1.0 degrees, without glare) and serum lutein.

The sequence and correlations between markers of lutein and zeaxanthin (dietary markers: F+V intake; status markers: their concentrations in serum [short-term marker] and MPOD [long-term marker]) and CT as the potential functional effect of lutein and zeaxanthin are summarized in figure 1. The degree of correlation (R^2) between MPOD and CS is higher without glare than under glare conditions in both age groups, being higher in the older group (2.9%-5.8% and 3.3%-7%, with and without glare, respectively) than in the younger group (0.1%-0.7% and 0.04%-1.3 %, with and without glare, respectively) (Figures in supplemental material).

Table 4 corresponds to the regression model (GELIN) used to evaluate the predictive value of sex, serum lutein concentration, lutein + zeaxanthin / cholesterol + TG in serum, F+V intake (every 100 g/day) and MPOD for CT at six visual angles of different degrees, with and without glare. Results from the multivariate regression analysis showed different correlations with CT, depending on the age group. In the older group, without glare (table 4), lutein in serum (at all the visual angles), MPOD (at medium and smaller angles: 1.6, 1.0, 0.7) and sex (at large and intermediate angles) were the main predictors of CT; with glare (table 4), the main predictors of CT were the same as in the absence of glare, but MPOD showed

significant associations at almost all the angles used. In the younger group, only F+V intake (every 100 g/day) was a predictor of CT without glare (at large angles; inverse association) and with glare (at large and intermediate angles; direct association). In the total sample, only MPOD, F+V intake and sex were predictors of CT, with and without glare, at every stimulus size.

4. Discussion

The present study focuses on the complete sequence of variables involved in the relationship between MPOD and visual function: dietary L+Z intake, the concentration of these carotenoids in blood, MPOD and, as final outcome, visual function, measured by CT. This approach was the strength of this study, and the limitations were mainly related to the optical and ocular variables of the participants - which were self-reported and, thus, not accurately controlled – and to the absence of gold standard techniques or test parameters to assess visual performance [6], as users of commercially available devices. This also makes it difficult to compare results across studies. Based on the findings, the hypothesis of this study is accepted, as the CT is influenced not only by MPOD, but by biochemical markers of the dietary intake of L+Z, as well. The results also reflect different dietary habits according to age.

The CT in the younger group is lower than in the older group, a finding that agrees with the results of previously published studies that show a decline of CS with age [8]. In our study, CT also differs according to light levels, being higher in the presence of glare, and these differences are more marked in the older group. MPOD is lower in the older than the younger group [23], as it also declines with age [30] (although some authors have reported no differences) [8]. This decline may be attributable to a decrease in the capacity to respond to

carotenoid consumption (in general, the intake is higher among older individuals) and, therefore, to defective capture of circulating carotenoids by the central retina [31].

MPOD shows significant inverse correlations with CT at every stimulus size, with and without glare, as well as with F+V intake (direct correlation) in the total sample. However, when we divide the sample according to age, CT, with and without glare, is correlated with MPOD only in the older group, in agreement with other studies [6, 8], as well as with serum L+Z concentration (a short-term biomarker of their status) [32, 33]. In contrast, in the younger group, there are correlations between CT and F+V intake (inverse correlations) and L+Z in serum only at the larger visual angles. It is difficult to explain that, whereas CT is correlated with the MPOD of those subjects whose MPOD is lower (older group), who, on the other hand, have higher serum lutein and zeaxanthin concentrations and higher F+V intake [23], no correlations are observed in the younger subjects, despite their higher MPOD values and higher CS. A strong correlation between the intake of those foods (but not dietary lutein and zeaxanthin intake) and MPOD has been reported [23, 34]. This suggests that, although fruit and vegetable intake are good markers of lutein and zeaxanthin intake [35, 36], these foods are important sources of other micronutrients and bioactive compounds that are also beneficial (e.g., fiber and polyunsaturated fatty acid intake are also directly related to MPOD) [34] and may have a role in visual function. However, F+V intake in the overall study sample is directly correlated with CT, and inversely associated with CT (without glare at some visual angles) in younger. We have no explanation for the direct association, as that would mean a worse CT. On the other hand, differences in serum lipid concentrations could be a determining factor in the relationship between MPOD and CT. MPOD is influenced by serum lutein (younger individuals) and by L+Z in relation to circulating lipids (older group) and, although these subjects had serum cholesterol levels within normal range, the older group had

higher cholesterol and low-density lipoprotein (LDL)-cholesterol than the younger group [23]. Moreover, potential oxidative modifications of LDL and high-density lipoprotein (HDL)-cholesterol that affect lipoprotein metabolism [37], and variations in the lipoprotein receptors that could be selective for different ratios of LDL to HDL [38], may have an effect on retinal pigment epithelial cells.

The MP is generally related to improvements in glare disability and visual performance [7, 15, 19], although the relationship between MPOD and visual performance seems to be age-dependent, as different correlations are observed in the two age groups in this study.

According to our findings, the MPOD was indirectly associated with the CT, with and without glare, at every size of stimulus, showing statistical significance in older subjects and with low coefficients. The highest R^2 found are 0.01 in younger and 0.06 in older subjects. On comparing our data with those from healthy young subjects (18-41 y), described by Loughman et al [6], who reported a $r=0.22$ as the strongest and most significant relationship between MPOD and CS (for intermediate light levels), the associations found in our young subjects are quite lower; however, the R^2 of the older participants is more comparable.

Besides the methodological differences between studies, it is intriguing to note that we found a statistically significant relationship in the older subjects, but not in the younger ones. Under glare conditions, better correlations are obtained at lower frequencies (large visual angles of 6.3, 4 and 2.5 degrees) in the older group, whereas weaker correlations are obtained at intermediate and high frequencies.

The limitations of the method of measuring the MP must be taken into account. Regarding that used in the present study, heterochromatic flicker photometry, as it is based on psychophysical methodology, differences in the exact point on which the subject focuses his

or her view to detect the flicker (border or center of the stimulus) could lead to a bias toward lower values [24]. In older subjects, although the percentages are usually small, more than one measurement is required before satisfactory results are obtained [39]. Thus, it is important that manufacturer's guidelines be well expressed and correctly followed to ensure reliability and reproducibility [40]. In general, significant correlations between variation in MPOD and immediate effects on visual function [33] have been reported, but as there are no gold standard techniques or test parameters to assess visual performance [6], results from the literature are difficult to compare.

MPOD, together with serum lutein, was found to predict CT, with and without glare, in the older group (45-65 y). This would be the target population for an improvement in vision quality through dietary means, given that a higher intake of a variety of fruits and vegetables and food supplements has been shown to produce an increase in serum lutein and in MPOD [12, 41], with no adverse effects reported to date at the concentrations supplied [42, 43]. Furthermore, F+V intake was identified as the only predictor in the younger group (without glare). Additional studies are required to better understand why those who could benefit from a higher intake of lutein were the older subjects, who already had higher fruit and vegetable intake and serum lutein concentrations, compared with the younger group.

Acknowledgments

This study was supported by a grant from the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación Tecnológica, Spain) (grant no. PS09/00681), and Rocío Estévez-Santiago is the recipient of a JAE-Predoc grant from the Consejo Superior de Investigaciones Científicas (CSIC), awarded under the program of the Junta para la Ampliación de Estudios,

co-financed by the European Social Fund. The authors wish to thank Laura Barrios and Belén Garzón from the CSIC for their support in the statistical study, ML García González for technical assistance and Martha Messman for editorial assistance.

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Figure 1. Sequence and correlations between dietary and status markers of lutein and zeaxanthin and contrast threshold.

Younger subjects

Older subjects

Curves show significant correlations in the younger group (solid curved lines) and older group (hatched curved lines).

^a Only at higher-degree visual angles.

Table 1. Contrast threshold at different degrees of visual angle, without and with glare ^a.

Visual angle of the stimulus (degrees)	Contrast threshold			
	Without glare ^b		With glare ^b	
	Aged 20-35 y ^c	Aged 45-65 y ^d	Aged 20-35 y ^c	Aged 45-65 y ^d
6.3	0.012 ± 0.000 (0.01) [0.012 , 0.013]	0.018 ± 0.001 (0.014) [0.016 , 0.020]	0.012 ± 0.000 (0.010) [0.012 , 0.013]	0.018 ± 0.001 (0.014) [0.016 , 0.020]
4.0	0.012 ± 0.000 (0.010) [0.011 , 0.012]	0.025 ± 0.002 (0.020) [0.021 , 0.03]	0.012 ± 0.000 (0.010) [0.011 , 0.013]	0.026 ± 0.002 (0.20) [0.021 , 0.031]
2.5	0.013 ± 0.001 (0.010) [0.012 , 0.014]	0.036 ± 0.003 (0.025) [0.030 , 0.042]	0.014 ± 0.001 (0.014) [0.013 , 0.016]	0.041 ± 0.004 (0.030) [0.034 , 0.048]
1.6	0.019 ± 0.001 (0.014) [0.016 , 0.020]	0.062 ± 0.005 (0.040) [0.051 , 0.072]	0.022 ± 0.001 (0.014) [0.019 , 0.024]	0.074 ± 0.006 (0.06) [0.062 , 0.090]
1.0	0.033 ± 0.002 (0.030) [0.029 , 0.036]	0.140 ± 0.011 (0.080) [0.114 , 0.157]	0.041 ± 0.003 (0.03) [0.035 , 0.048]	0.160 ± 0.012 (0.11) [0.140 , 0.190]
0.7	0.075 ± 0.006 (0.060) [0.063 , 0.090]	0.273 ± 0.016 (0.230) [0.241 , 0.305]	0.09 ± 0.008 (0.08) [0.08 , 0.11]	0.310 ± 0.020 (0.320) [0.270 , 0.340]

^a Values are expressed as means ± SD, (median), [95%CI]. n= 216 eyes.

^b Mann- Whitney U test. Significant differences between the two age groups, with and without glare, at any stimulus size (p<0.000). ^c Differences in CT, with and without glare, at 2.5 degrees, p= 0.004; at 1.6 and 1.0 degrees, p = 0.001 and at 0.7 degrees, p=0.000. ^d Differences in CT, with and without glare, at 2.6 ° p= 0.006; at 1.6°, 1.0 ° and 0.7° p= 0.000

Table 2. Macular pigment optical density, dietary (fruit and vegetable intake) and biochemical variables (lutein and lutein+zeaxanthin / cholesterol + triglycerides in serum) of subjects.

Subjects (n=108)		
	Aged 20-35 y	Aged 45-65 y
MPOD (density units)	0.37 ± 0.014 (0.36) [0.34 , 0.40]	0.33 ± 0.016 (0.32) [0.29 , 0.36]
Fruit + vegetable intake (g/day)	388.4 ± 18.2 (410.3) [352.2 , 424.5]	610.3 ± 28.2 (577.3) [554.4 , 666.2]
Lutein^a µg/dl, serum	10.9 ± 0.50 (9.90) [10.0 , 11.86]	14.76 ± 0.64 (13.04) [13.50 , 16.02]
Lutein + zeaxanthin / cholesterol + triglycerides (µg/mg, serum)	0.06 ± 0.002 (0.050) [0.05 , 0.06]	0.06 ± 0.002 (0.054) [0.057 , 0.068]

Values are means ± SD, (median) and confidence interval [95% CI]. N= 108. Data from Olmedilla-Alonso et al [21].

^a Lutein means ± SD: 0.192 ± 0.009 (aged 20-35y) and 0.259±0.011 (aged 45-65y) µmol/L

Table 3. Correlations of contrast threshold (without and with glare) with MPOD, serum lutein+zeaxanthin / cholesterol+triglycerides, serum lutein and fruit + vegetable intake of subjects^a.

	Aged 20–35 y ^b		Aged 45-65 y		Total sample ^c			
	L+Z / cholesterol+TG	F+V	MPOD	L+Z / cholesterol+TG	Lutein	MPOD	L+Z/cholesterol+TG	F+V
<i>Without glare</i>								
6.3	-0.215 (0.025)	-0.299 (0.002)	- 0.238 (0.013)			- 0.152 (0.026)		
4.0		-0.196 (0.042)	- 0.219 (0.023)		-0.220 (0.022)	- 0.160 (0.018)		0.208 (0.002)
2.5			- 0.278 (0.004)		-0.209 (0.030)	- 0.238 (0.000)		0.218 (0.001)
1.6			- 0.333 (0.000)			-0.213 (0.002)		0.180 (0.008)
1.0			- 0.324 (0.001)	-0.207 (0.032)	-0.224 (0.020)	- 0.245 (0.000)		0.176 (0.010)
0.7			- 0.280 (0.003)			- 0.213 (0.002)		0.213 (0.002)
<i>With glare</i>								
6.3			- 0.248 (0.010)			- 0.182 (0.007)		0.264 (0.000)
4.0			- 0.216 (0.025)			- 0.170 (0.012)		0.223 (0.001)
2.5			- 0.287 (0.003)			- 0. 181 (0.008)		0.260 (0.000)
1.6			- 0.283 (0.003)			- 0.177 (0.009)		0.265 (0.000)
1.0			- 0.269 (0.005)			-0.205 (0.002)		0.232 (0.001)
0.7			- 0.241			- 0.176		0.202

L+Z / cholesterol+TG		0.262 (0.006)	0.170 (0.012)		
Lutein	0.844 (0.000)	0.204 (0.034)	0.864 (0.000)	0.830 (0.000)	
F+V	0.382 (0.000)	0.350 (0.000)	0.233 (0.015)	0.171 (0.012)	0.326 (0.000)

^a Spearman correlations. N=216 eyes. Units: MPOD (density units), serum lutein+zeaxanthin / cholesterol + triglycerides (µg/mg), serum lutein (µg/dl) and fruit + vegetable intake (g/day). (*p* value). Correlations between F+V intake and lutein (serum) and between F+V intake and MPOD were reported reviously [23].

^b Aged 20-35 years: no significant correlations found between MPOD and CT. Serum lutein and F+V intake correlation: 0.447 (0.000). ^c

Total sample: Serum lutein and F+V intake correlation: 0.355 (0.000).

F+V, fruit and vegetable; L+Z, lutein plus zeaxanthin; MPOD, macular pigment optical density; TG, triglycerides.

Table 4. Association between contrast threshold, without and with glare, and MPOD, biochemical and dietary factors ^a and sex of subjects.

Visual angle (degrees)	6.3	4.0	2.5	1.6	1.0	0.7
<i>WITHOUT GLARE</i>						
<i>20 to 35-year-old group</i>						
Constant	-4.19 (0.11) [-4.39 , -3.98] 0.000	-4.50 (0.09) [-4.68 , -4.33] 0.000	-4.25 (0.11) [-4.48 , -4.03] 0.000	-4.02 (0.16) [-4.33 , -3.70] 0.000	0.036 0.01) [0.02 , 0.05] 0.000	0.10 (0.02) [0.06 , 0.15] 0.000
F + V intake	- 0.04 (0.02) [- 0.07 , -0.07] 0.020	-0.03 (0.01) [-0.05 , -0.001] 0.044				
<i>WITH GLARE</i>						
<i>20 to 35-year-old group</i>						
Constant	-4.49 (0.11) [-4.71 , -4.28] 0.000	-4.46 (0.11) [-4.67 , -4.25] 0.000	-0.01 (0.02) [0.01 , 0.02] 0.000	0.02 (0.01) [0.01 , 0.030] 0.000	0.05 (0.01) [0.03 , 0.08] 0.000	0.10 (0.03) [0.05 , 0.16],0.000
F + V intake	0.05 (0.016) [0.02 , 0.08] 0.002	0.04 (0.02) [0.01 , 0.07] 0.014	0.001 (0.0003) [0.000 , 0.002] 0.004	0.002 (0.0007) [0.000 , 0.003] 0.031		
Lutein				-0.001 (0.0004) [-0.002 , -0.00] 0.036		

WITHOUT GLARE		45 to 65-year-old group				
Constant	-4.00 (0.23) [-4.45 , -3.55] 0.000	-3.99 (0.30) [-4.58 , -3.40] 0.000	-3.23 (0.32) [-3.85 , -2.62] 0.000	-2.41 (0.32) [-3.04 , -1.78] 0.000	0.20 (0.05) [0.11 , 0.30] 0.000	0.34 (0.08) [0.19 , 0.49] 0.000
MPOD				-1.36 (0.46) [-2.27 , -0.46] 0.003	-0.16 (0.07) [-0.30 , - 0.02] 0.030	-0.28 (0.11) [-0.49 , -0.06] 0.013
L+Z/cholesterol+TG				10.75 (5.27) [0.417 , 21.07] 0.041		
Lutein	-0.04 (0.02) [-0.07 , -0.01] 0.016	-0.04 (0.02) [-0.08 , -0.002] 0.040	-0.06 (0.022) [-0.10 , -0.02] 0.005	-0.07 (0.02) [-0.12 , -0.02] 0.004	-0.006 (0.003) [-0.01 , 0.00] 0.056	-0.009 (0.005) [-0.02 , 0.00] 0.060
Sex		0.44 (0.12) [0.20 , 0.68] 0.000	0.39 (0.13) [0.13 , 0.65] 0.004			
WITH GLARE		45 to 65-year-old group				
Constant	-4.28 (0.30) [-4.69 , -3.87] 0.000	-4.11 (0.30) [-4.70 , -3.51] 0.000	0.04 (0.02) [0.01 , 0.07] 0.022	0.08 (0.03) [0.21 , 0.14] 0.008	0.20 (0.06) [0.09 , 0.31] 0.001	0.43 (0.08) [0.27 , 0.58] 0.000
MPOD	-0.89 (0.29) [-1.47 , -0.32] 0.002			-0.08 (0.04) [-0.17 , 0.003] 0.059	-0.15 (0.08) [-0.32 , 0.01] 0.063	-0.21 (0.11) [-0.43 , 0.01] 0.062
F + V intake	0.01 (0.016) [0.001 , 0.06] 0.045					
L+Z /cholesterol + TG		10.05 (4.62) [0.99 , 19.11] 0.03				
Lutein		-0.05 (0.20) [-0.09 , -0.01] 0.008			-0.01 (0.004) [-0.02 , -0.001] 0.026	-0.01 (0.01) [-0.02 , -0.00] 0.045
Sex	0.22 (0.09) [0.04 , 0.39] 0.014	0.52 (0.17) [0.27 , 0.77] 0.000	0.01 (0.01) [0.000 , 0.03] 0.042			
WITHOUT GLARE		TOTAL SAMPLE				
Constant	-4.33 (0.14) [-4.60 , -4.06] 0.000	-4.55 (0.19) [-4.92 , -4.19] 0.000	-4.07 (0.21) [-4.49 , -3.65] 0.000	-3.49 (0.23) [-3.94 , -3.03] 0.000	0.07 (0.03) [0.02 , 0.13] 0.007	0.12 (0.05) [0.03 , 0.21] 0.007
MPOD	-0.52 (0.19) [-0.90 , -0.14] 0.007	-0.75 (0.26) [-1.26 , -0.25] 0.004	-1.36 (0.31) [-2.00 , -0.76] 0.000	-1.86 (0.33) [-2.50 , -1.21] 0.000	-0.17 (0.04) [-0.25 , -0.10] 0.000	-0.29 (0.07) [-0.41 , -0.16] 0.000
F+ V intake	0.03 (0.01) [0.01 , 0.06] 0.004	0.08 (0.02) [0.05 , 0.11] 0.000	0.07 (0.02) [0.04 , 0.11] 0.000	0.08 (0.02) [0.04 , 0.12] 0.000	0.01 (0.00) [0.00 , 0.01] 0.004	0.02 (0.00) [0.01 , 0.03] 0.000
L+Z/chol+TG		-6.28 (2.74)	-6.23 (3.21)			

		[-11.63 , -0.92] 0.022	[-12.52 , 0.06] 0.052			
Sex	0.16 (0.06) [0.04 , 0.28] 0.009	0.37 (0.08) [0.21 , 0.53] 0.000	0.36 (0.10) [0.18 , 0.55] 0.000	0.31 (0.11) [0.10 , 0.52] 0.003	0.03 (0.01) [0.00 , 0.05] 0.027	0.05 (0.02) [0.01 , 0.10] 0.029
WITH GLARE	TOTAL SAMPLE					
Constant	-4.50 (0.12) [-4.74 , -4.26] 0.000	-4.55 (0.19) [-4.92 , -4.18] 0.000	0.02 (0.01) [-0.00 , 0.03] 0.090	0.03 (0.02) [-0.01 , 0.06] 0.100	0.07 (0.03) [0.01 , 0.13] 0.030	0.17 (0.05) [0.08 , 0.27] 0.000
MPOD	-0.76 (0.17) [-1.10 , -0.42] 0.000	-0.96 (0.27) [-1.49 , -0.43] 0.000	-0.04 (0.01) [-0.07 , -0.02] 0.000	-0.01 (0.02) [-0.13 , -0.04] 0.000	-0.19 (0.05) [-0.28 , -0.10] 0.000	-0.28 (0.07) [-0.41 , -0.14] 0.000
F+ V intake	0.06 (0.01) [0.04 , 0.08] 0.000	0.06 (0.02) [-0.03 , -0.09] 0.000	0.00 (0.00) [0.00 , 0.00] 0.000	0.01 (0.00) [0.00 , 0.01] 0.000	0.01 (0.00) [0.00 , 0.02] 0.000	0.02 (0.00) [0.01 , 0.03] 0.000
Sex	0.18 (0.06) [0.08 , 0.29] 0.001	0.40 (0.08) [0.24 , 0.57] 0.000	0.01 (0.00) [0.00 , 0.02] 0.003	0.02 (0.01) [0.01 , 0.03] 0.005	0.04 (0.01) [0.01 , 0.06] 0.015	

^a Only statistically significant results are shown. β Coefficient and (standard error), [95% CI] and p from generalized linear models (GLM, TWEEDIE distribution and LINK=LOG).

Units: MPOD (density units), biochemical ($\mu\text{g}/\text{mg}$ and $\mu\text{g}/\text{dl}$), dietary factors (F + V intake, 100 g/day).

CI, confidence interval; F+V, fruit and vegetable; L+Z, lutein plus zeaxanthin; MPOD, macular pigment optical density; TG, triglycerides.

Figure 2. The relationship between MPOD and CS, with and without glare, at six levels of stimuli in the two age groups.

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