Predictors of macular pigment and contrast threshold in normolipemic subjects aged 45-65

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Research

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Predictors of macular pigment and contrast threshold in normolipemic subjects aged 45-65

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Abstract

Objective: The dietary carotenoids lutein and zeaxanthin are transported in the bloodstream by lipoproteins and selectively captured in the retina where they constitute macular pigment. There are no lutein and zeaxanthin dietary intake recommendations nor desired blood/tissue concentrations for the general population. The aim of this study was to determine the lutein and zeaxanthin dietary intake, their serum concentrations, lipid profile, macular pigment optical density (MPOD) and the contrast sensitivity (CT), as visual outcome in normolipemic subjects age 45-65 (n=101).

Methods: MPOD, L and Z in serum and dietary intake were determined using heterochromatic flicker photometry, high-performance liquid chromatography and 3-day food records. CT was measured with the CGT-1000 Contrast Glaretester at six stimulus sizes, with and without glare.

Results: Lutein and zeaxanthin serum concentrations (median): 0.361 and 0.078 µmol/L. Lutein+zeaxanthin intake: 1.1 mg/d (median). MPOD: 0.34 du. Lutein+zeaxanthin intake correlates with their serum concentrations (ρ=0.333, *p*=0.001), which in turn correlates with MPOD (ρ=0.229, *p*=0.000) and with the fruit and vegetable consumption (ρ=0.202, *p*=0.001), but not with the lutein+zeaxanthin dietary intake. HDL-cholesterol correlated with lutein+zeaxanthin serum (ρ=0.253, *p*=0.000) and with CT under glare conditions (ρ range: 0.016–0.160). MPOD predictors: serum lutein+zeaxanthin, lutein+zeaxanthin/HDL-cholesterol and HDL-cholesterol (R^2=15.9%). CT predictors: MPOD and sex (β coefficients ranges: -0.950,-0.392; -0.134,-0.393, respectively).

Conclusion: There were correlations at all points in time in this sequence between lutein+zeaxanthin intake and the visual outcome and, HDL-cholesterol played a relevant role.

Keywords. Macular pigment optical density; lutein; zeaxanthin; HDL-cholesterol; LDL-cholesterol; contrast threshold; dietary intake; serum concentrations.

1. Introduction

Macular pigment (MP), in the central region of the retina, is made up of lutein and zeaxanthin, major carotenoids in the human diet, and meso-zeaxanthin, which is of non-dietary origin. Of the many carotenoids present in the diet and transported in the bloodstream to tissues by lipoproteins, only these are captured by the retina where they act as blue light filters, antioxidant and anti-inflammatory agents [1], and enhance gap junctional communication [2], which, in the retina, is important for light processing [3]. An adequate nutritional status (lutein and zeaxanthin serum concentrations and macular pigment optical density [MPOD]) is associated with a lower risk of several age-related diseases, particularly age-related eye diseases [4-6].

Lutein and zeaxanthin are among the food components that have proven to be effective in lowering the risk and /or progression of age-related macular degeneration (ARMD), a major cause of blindness in the elderly population [7]. There has recently been an increase in research looking into the potential of lutein in slowing down age-related decline and how to recover damaged cognitive functions [5]. Up to 10 mg lutein/day has been recommended for people at risk or in the intermediates stages of age-related macular disease [8], equivalent to approx. 100g spinach/day. However, there are no lutein and zeaxanthin dietary intake recomendations for general population, nor are there desired blood or tissue concentrations despite ample information rearding their status, dietary intake and biochemical markers. This is partly due to the great deal of variability in carotenoid bioavailability which depends on several host and food related factors [9,10]. However, reference recommendations have recently been claimed for lutein intake as regards optimal ocular health and lowering the risk of chronic eye disease as this compound meets a

series of global criteria established for non-essential bioactive components regarding diet and health [11].

The macula is an important target tissue to consider when developing dietary recommendations for carotenoids, MPOD being the most accessible marker for xanthophyll concentration in the macula [10]. Moreover, evidences have been found supporting the effect of dietary supplements containing macular pigment carotenoids in improving visual performance in patients and in controls groups [6]. However, few studies have focused on the visual outcomes of lutein and zeaxanthin dietary intake and status (serum concentrations and MPOD) with visual outcomes (eg. contrast sensitivity, glare sensitivity, photostress recovery, visual acuity) in adults with healthy eyes [3]. In a recent study, we described age-specific correlations for the MPOD and the impact of lutein+zeaxanthin in relation to circulating lipids in apparently healthy subjects [12] and, MPOD was associated with contrast sensitivity (CT) in older subjects [13]. In this study the aim was to assess lutein and zeaxanthin dietary intake in a larger sample, their serum concentrations, lipid profile, MPOD value and contrast sensitivity in normolipemic subjects age 45-65.

2. Materials and methods

2.1. Design

An analytical observational design was employed to analyse dietary intake, status of lutein and zeaxanthin (serum concentrations and MPOD) and serum lipid profile to asses their predictive value on MPOD and on visual function as determined by CT in subjects age 45 to 65.

2.2 Participants

101 volunteers (77 women and 24 men) age 45 to 65 y (mean ± SD: 54.4 ± 0.6 y) were enrolled in a cross-sectional study. Participants were selected from among those interested after learning of the study through advertisements at different universities, research centers and notice boards. The inclusion of an equal number of women and men was not posible due to the lack of interest on the part of men.

78 The inclusion criteria were normal cholesterolemia (upper limit *ca.* 6.22 mmol/L), BMI ≥20 and
79 ≤30 kg/m2, mixed diet (no avoidance of any food groups). Exclusion criteria (self reported):
80 consumption of dietary supplements, surgery for myopia (within the previous year), cataracts or macular
81 degeneration, use of drugs or phytosterol-enriched beverages/foods to control cholesterolaemia,
82 consumption of n-3 fatty acid-enriched food products and chronic diseases that can affect carotenoid or
83 lipid metabolism (i.e. diabetes, cardiovascular difsease). Of the one hundred and twenty one individuals
84 who showed their interest in participating in the study, five were excluded because of his/her BMI, three
85 because they were taking food supplements (omega-3, lutein+zeaxanthin), three because their age, two
86 because of statin consumption, two because of ocular chronic diseases, three without giving any
87 explanation, one because followed a vegan diet and one because of gastric bypass.

88 **2.3. Procedures**

89 The volunteers included in this cross-sectional study underwent blood sampling, assessment of
90 the MPOD and of the visual function, measured by the CT, and 3-day food records. The subjects were
91 enrolled over the course of an entire year (spring and summer: 49 and during the fall and winter: 62).
92 Blood samples were collected after overnight fast (at least 9 hours).

93 This study was conducted in accordance with the guidelines laid down in the Declaration of
94 Helsinki and all procedures involving human subjects were approved by the Ethical Committee of
95 Research with Drugs of the Hospital Universitario Puerta de Hierro Majadahonda of Madrid, Spain (acta
96 nº 03.17, dated 13 February 2017) and by the Bioethic Subcommittee- Ethics Commitee (CSIC) (dated
97 21 February 2017). Written informed consent was obtained from all subjects.

98 **2.4. Lutein, zeaxanthin and lipid analysis in blood**

99 Lutein and zeaxanthin levels were determined by high performance liquid chromatography
100 (HPLC) using a system consisting of a model 600 pump, a Rheodyne injector and a 2998 photodiode
101 array (PDA) detector (Waters, Milford, MA, USA). The system included a C30 YMC column (5 µm,
102 250×4.6 mm i.d.) (Waters, Wilmington, MA) with a guard column (Aquapore ODS type RP-18). The
103 mobile phase, in a linear gradient, was metanol (MeOH) with 0.1% triethylamine (TEA)/metyl ter-butyl

ether (MTBE) from 95:5 to 70:30 in 25 min, to 35:65 in 25 min, to 95:5 in 10 min and maintaining this proportion for 8 min. The flow rate was 1 mL min−1, and detection was performed at a wavelenght of 450 nm. All chromatograms were processed using Empower 2 software (Waters, Milford, MA, USA). Identification was carried out by comparing the retention times with those of authentic standards and on-line UV-VIS spectra.

Carotenoid extraction was performed on serum samples using a slight modification of a previously published method [14]. Briefly, 600 µl of serum was added to 600 µl of ethanol, vortexed and extracted twice with 1200 µl of hexane: dichloromethane (5:1) stabilized with 0.1 g/L butylated hydroxyltoluene. Organic phases were pooled, evaporated to dryness under nitrogen atmosphere and reconstituted with 200 µl of a solution of metanol : methyl ter-butyl ether (1:1) and injected (50 µl) onto the HPLC system.

Methanol and metyl ter-butyl ether from Honeywell Research Chemicals, triethylamine and butylated hydroxytoluene from Sigma-Aldrich, hexane and dichloromethane were supplied by Lab-Scan and Panreac (Barcelona, Spain), respectively. Lutein (xanthophyll from marigold) and zeaxanthin were obtained from Sigma-Aldrich.

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 $E_{1cm}^{1\%}$ values and wavelengths used were: lutein, 2550 at 445 nm; zeaxanthin, 2540 at 450 nm. Working solutions were obtained from different volumes of the standard solutions dissolved in methanol:metyl ter-butyl ether (1:1 v/v). The concentrations of the carotenoids in the curve were: 0.27-1.36 µg mL^{-1} for lutein (R^2=0.999) and 0.03-0.15 µg mL^{-1} for zeaxanthin (R^2=0.999).

Blood total cholesterol were analyzed by enzimatic assay and and high-density lipoprotein (HDL) cholesterol by catalasa assay kit (ADVIA-XPT, Siemens). The low-density lipoprotein (LDL)-cholesterol level was calculated with the Friedewald equation [15]. Serum triglycerides (TG) were determined colorimetrically using the Fossati reaction with a Trinder type reaction final (GPO Trinder). Glucose was analyzed by the hexokinase method.

2.5. Dietary intake assessment

Recent dietary intake was evaluated using 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. The participants underwent a face-to-face encounter with a specialized interviewer for the first recall. The amounts consumed were estimated in units (fruits), portions or household servings [16]. 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 [17] included in a software application for the calculation of dietary intake of individual carotenoids [18]. To evaluate the energy intake, we employed a food composition table included in the software DIAL® [16].

2.6. Macular pigment optical density (MPOD) assessment

Macular pigment optical density 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. [19]. The test consists of two stages for central and peripheral viewing, and the subjects were required to press a response button as soon as they detect flicker. The subjects started by fixating the central stimulus, a 1-degree central target (flicker rate is initially set to 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.

2.7. 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 anular stimuli with diameters ranging from 6.3° to 0.7° 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/m2. The specifications selected for the presentation of the stimulus were: presentation duration, 0.2 seconds; presentation interval, 2 seconds; test luminance with glare, 40,000 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.

2.8. Statistical analysis

Data are expressed as means and standard deviations, medians and 95% confidence intervals (CI). Lutein and zeaxanthin in serum and diet and TG did not follow a normal distribution (assessed by Kolmogorov-Smirnov test) and U Mann-Whitney test was used for comparison between sexes.

Correlations among variables in serum, diet, MPOD and CT were established using Spearman's rho correlation coefficient. For he analysis of the correlations among variables and for the generalized linear model we used a total sample of 145 subjects, as to the 92 from this study we incorpored data from 53 subjects, with the same characteristics, from a preliminary study previously published [12,13].

Multiple linear regression analysis was carried out using backward elimination as a model selection procedure, with MPOD as dependent variable and as independent variables the lipids concentrations, the lutein and xanthophylls expressed in relation to lipids concentrations (cholesterol, HDL-cholesterol, LDL-cholesterol, serum lutein+zeaxanthin, lutein+zeaxanthin/HDL-, lutein+zeaxanthin/LDL-, lutein+zeaxanthin/cholesterol+TG) and, fruit intake and vegetable intake.

The contrast sensitivity 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 (TWEEDIE distribution and LINK = LOG), with CT (6 levels of stimuli, visual angle degrees) as the dependent variable and with fixed factor (sex) and covariates (serum lutein, HDL-cholesterol, LDL-cholesterol, cholesterol+ TG, MPOD and fruit +vegetables).

All reported P-values are based on a two-sided test and compared to a significance level of 5%. IBM SPSS v.25 Statistics software was used.

3. Results

Table 1 shows subject characteristics, including the lipid concentrations as confounding factor for the interpretation of the lutein and zeaxanthin status. There were no differences in the range of age, the body mass index or blood pressure and, regarding lipid profile, only the HDL-cholesterol showed differences between sexes. Most of the subjects did not smoke (81 out of 92). Although 101 subjects participated in this study, nine of them were excluded because of serum lutein concentration above 0.63 µmol/L, concentration considered as that attainable by dietary means in normolipemic subjects [4,12]. Higher concentration is compatible with levels attained by consuming lutein food supplements, despite the fact that candidates were asked about such consumption as this was considered an exclusion criteria. This concentration represented the 92 centil in this study.

The lutein and zeaxanthin concentrations in serum and in dietary intake, MPOD, energy and fruit and vegetable consumption are shown in table 2. Women exhibited significantly higher lutein concentrations in serum and higher MPOD levels but there were no differences in lutein and zeaxanthin intake or fruit and vegetable consumption. Serum lutein concentration related to LDL-cholesterol and to cholesterol plus TG wer also higher in women than in men.

Table 3 shows the significant correlations among serum lutein and zeaxanthin concentrations, their dietary intake and consumtion of fruit and vegetables. Serum lutein and zeaxanthin also correlated with the serum cholesterol and HDL-cholesterol, but not LDL-cholesterol. The same lipid variables correlated significantly with sex (HDL-cholesterol ρ=0.421, p=0,000; cholesterol ρ=0.122, p=0.039). Sex also correlated with serum concentrations of lutein (ρ=0.216, p=0.000), zeaxanthin (ρ=0.130, p=0.027), lutein plus zeaxanhin (ρ=0.187, p=0.001), lutein plus zeaxanthin/colesterol+TG (ρ=0.206, p=0.000). The highest correlations were found between the lutein+zeaxanthin intake and fruit and vegetable consumption.

206 The CT data for each of six different degrees of visual angle, with and without glare, are shown in
207 table 4. In general, these values were higher with glare than without glare. The lower the CT, the higher
208 the contrast sensitivity level at which a subject was able to detect each spatial frequency. At most of the
209 visual angle of the stimulus, CT correlates with the MPOD (table 5). For its part, the MPOD correlates
210 significanty with serum lutein and zeaxanthin concentrations and with fruit and vegetable consumption,
211 but not with the lutein+zeaxanthin dietary intake (table 5).

212 None of the lipid profile variables correlated significantly with MPOD. However, HDL-cholesterol
213 did correlate with CT, only with glare, at the large and medium stimulus sizes (at 6.3º: 0.160, p=0.06; at
214 4.0º: 0.140, p= 0.017; at 2.5º: 0.116, p=0.048).

215 Table 6 shows the regression model used to evaluate the predictive value of serum lutein and
216 zeaxanthin expressed in relation to serum lipids, fruit and vegetable consumption and sex, on the MPOD
217 value. MPOD did not show diferences between sexes in the total sample. The table only shows results
218 that do not include zero in the confidence interval. Serum lutein plus zeaxanthin, total concentration and
219 expressed in relation to HDL-cholesterol and HDL-cholesterol, were the main predictors of MPOD. The
220 R^2 was 15.9% for the entire sample and, broken down by sex, was 17.6% and 23.2% for women and
221 men, respectively. If the concentration of luein and zeaxanthin expressed in relation is not considered,
222 the predictive value is only 8.4%, serum lutein being the main predictor.

223 Table 7 shows the results of the regression model (GELIN) used to assess the predictive value of
224 sex, lutein serum concentration, lutein+zeaxanthin/cholesterol+TG, MPOD and fruit and vegetable
225 consumption for CT at six visual angles of different degress, with and without glare. MPOD and sex
226 were predictors of CT at all visual angles and the main predictors of CT were the same with and without
227 glare, although not at all visual angles as MPOD was only at medium and smaller angles (2.5º, 1.6º, 1.0º
228 and 0.7º) and sex was not a predictor at 0.7º.

229

230 **4. Discussion**

MPOD is generally associated with lutein and zeaxanthin serum concentrations and, to a lesser degree, with the intake of lutein and zeaxanthin or the intake of their major dietary contributors. Many factors come into play here such as sex, age, body mass index, smoking status, circulating lipids and carotenoid intake patterns [10], to name few, and these should be taken into accunt in order to draw accurate comparisons between studies. It is also important to know not only how to modify MPOD by means of diet, but also the degree to which MPOD correlates to vision in apparently healthy subjects. With this study we aimed to deepen in the age-specific correlation for the MPOD with dietary and serum lutein, zeaxanthin and lipid concentrations we described previously in subjects 45-65y [12,13]. Subjects from the two studies had identical characteristics in terms of age, body mass index, and lipid concentration. In this study, serum lutein and zeaxanthin concentrations were higher than in the previous one (lutein median concentration: 0.361 *vs* 0.0242 µmol/L and zeaxanthin median concentration: 0.078 *vs* 0.048 µmol/L). This could be due to the higher dietary intake of lutein+zeaxanthin (median value 1089 *vs* 679 ug/d) and of the major contributors to their dietary intake (median fruit intake 323 *vs* 269 g/d and median vegetable intake: 342 vs 291 g/d). MPOD was slightly higher in this study (0.34 *vs* 0.32 du) but no differences between sexes were found in the total sample.

Mean serum lutein plus zeaxanthin in this study (0.477 µmol/L) is above the mean (0.330 µmol/L) but in the 0.199 – 0.561 µmol/L range of serum lutein and zeaxanthin and the mean dietary intake of lutein and zeaxanthin (1.5 mg/d) also falls in the 0.06 – 4.84 mg/d range (mean 2.2 mg/d) described in those studies [10], but is much lower than the 6 mg of lutein/day associated with decreased risk of several chronic diseases [20,4]. The serum lutein and zeaxanthin concentration found in this study is also lower than that associated with lower risk in epidemiological studies and with improvement in physiological functions and thus, quality of life of the subjects (> 0.63 µmol/L) [4].

Dietary intake and status markers are the first elements to be correlated in any study looking into the relationship between diet and health. Specially, regarding lutein and zeaxanthin, two status markers can be assessed: serum concentrations and MPOD as a short- and long-term markers, respectively. There was a similar / high correlation between dietary intake and serum concentrations (r=0.333) compared to

that found in a systematic review of more than a hundred validation studies on the correlation between lutein/zeaxanthin dietary intake and plasma levels, where r was 0.29 ($_{95\%}$CI: 0.26, 0.32) [20]. Interestingly, the serum concentration of lutein and zeaxanthin correlated significantly with fruit consumption (r=0.273) but not with the vegetable consumption, despite the fact that the amount of lutein+zeaxanthin supplied by vegetables is much greater than that supplied by fruits as shown by national surveys in Spain, for instance [21] and when assessed in small samples of adults (approximately eight times greater) [12]. This could partially be due to the fact that these xanthopylls are found in fruits in ester forms, which bioavailability is equivalent or even higher than that of free carotenoids [22]. However, dietary intake of lutein and zeaxanthin (total intake and expressed per 1000 Kcal) showed a higher level of correlation with vegetable as opposed to fruit consumption.

Lutein and zeaxanthin are transported by lipoproteins in the blood, which are mainly located on the surface and therefore, the transfer between lipoproteins might be easier for these xanthophylls than for the more apolar carotenoids and therefore, they could be evenly distributed between LDL and HDL fractions [9,23]. However, in this study serum lutein and zeaxanthin correlated with cholesterol and HDL-cholesterol, but not with LDL-cholesterol, which is compatible with the majority presence of these carotenoids in HDL-cholesterol compared to LDL-cholesterol described in several studies [25-28]. Moreover, there is a selective deposit of lutein and zeaxanthin in the eye, in the macular pigment, and it is believed to be regulated by binding proteins [9]. As in other studies with subjects of the same age group [12,29], MPOD correlated with serum lutein and zeaxanthin and also when they are expressed rin relation to lipids as reported in our previous study [12]. It also correlates with fruit and vegetable consumption [12,30]. We did not find any correlation between MPOD and serum cholesterol or lipoproteins, but did with lutein and zeaxanthin serum concentration expressed in relation to lipoproteins. However, a relationship between MPOD and cholesterol or lipoproteins has been reported in some studies with healthy subjects although not in others. For instance, MPOD was associated with HDL-cholesterol in young healthy subjects (mean age: 23) [31] but no associations were observed in other studies in subjects with wider age ranges, 21-66 [28] and 18-75 [32]. There are differences between

studies that may account for inconsistencies in results such as sample size, MP measurements methods and, two other aspects that are not generally considered: age group and cholesterolemia, both of which are particularly significant factors [12].

In multivariate regression analysis performed to assess the predictive value of serum lutein and zeaxanthin concentration, serum lipids, sex and fruit and vegetable consumption, only a direct association of serum lutein and zeaxanthin and an inverse associations of lutein+zeaxanthin/HDL-cholesterol and of HDL-cholesterol correlated with MPOD. This association between serum xanthophylls concentration and MPOD has been widely described and the lipoprotein profile is likely to affect MP levels, although the mechanisms controlling the deposition and stabilization of MP are not fully understood [10]. In this study a high HDL-cholesterol serum concentration is associated with lower levels of MPOD and, high HDL-cholesterol is associated with a higher risk of ARMD [33,34]. However, conflicting associations between circulating lipoprotein concentrations and ARMD have been described, in part due to the changes that composition and biological properties of the HDL particles undergo under different physiological and pathological conditions [35].

Furthermore, lutein and zeaxanthin serum concentrations, MPOD and their role in ocular health should not be considered independently of circulating lipids which have an impact on their presence in the bloodstream and in tissue uptake. Although lutein and zeaxantin are both transported in HDL, a selective uptake of zeaxanthin from HDL-cholestol by retinal pigment epithelium has been described [36]. The coefficient of determination of MPOD for lutein and zeaxanthin serum concentration and when expressed in relation to HDL-cholesterol in this study was 15.9% for the entire sample (23.2% in men and 17.6% in women), which is lower than the 29.7% obtained in the previous study [12]. However, in both cases lutein and zeaxanthin serum concentrations and also when expressed in relation with lipids were the determining factors. Therefore, for a better interpretation of the results, lutein and zeaxanthin concentrations are insufficient. They must be expressed as a function of the different lipid fractions because, as can be observed in this study, with the same lutein and zeaxanthin serum concentration

higher HDL-cholesterol could lead to a lower lutein plus zeaxanthin and HDL-cholesterol ratio and to higher MPOD.

MPOD can optimize visual performance because of its pre-receptoral absorption of blue light and the subsequent attenuation of the effects of chromatic aberration and the adverse effect of light scatter [37-39]. Increased MPOD has been associated with improved visual function in patients with early stage AMD [40] and in control subjects [41], just as higher serum lutein and zeaxanthin was related with improved visual function in patients with cataracts [42]. However, most studies evaluating lutein and zeaxanthin intake and visual outcomes focus on MPOD as the measure of interest, although there are a number of studies with other visual outcome indicators (contrast sensitivity, glare sensitivity, photostress recovery, visual acuity) which need to be thoroughly studied in healthy individuals with a varied diet [3]. In a previous study including subjects with the same characteristics, we described MPOD and serum lutein concentration as predictors of CT, with and without glare, only in subjects within this age group but not in younger subjects (25-45 y) and found that, sex had a bearing on CT values in the older group [13]. In that study, MPOD was associated with CT, without glare, at low visual angles and, with glare, at high visual angle (6.3º). In this study, CT and serum lutein concentrations did not correlate, but we did find a stronger associations between MPOD and CT at all visual angles, without glare, and at nearly all visual angles, with glare.

5. Conclusions

In the time sequence between dietary intake of lutein and zeaxanthin and that of their major dietary intake contributors (mainly fruits) and the visual outcome, significant correlations were found at all points in time in these normolipemic subjects age 45-65 with no ocular diseases. Circulating lipids play an important role in this process as HDL-cholesterol (but not LDL-cholesterol) is associated with serum lutein and zeaxanthin concentration and with the CT. Serum lutein and zeaxanthin concentration related to HDL-cholesterol is a predictor of MPOD, which, in turn, determines CT in these normolipemic subjects age 45-65 who are the target population for food supplements marketed to improve vision

quality and chronic eye disease risk reduction and which could benefit from recommendations regarding carotenoid intake or desired blood or tissue concentrations to improve ocular health.

Ethical Approval and consent to participate

This study was approved by the Ethical Committee of Research with Drugs of the Hospital Universitario Puerta de Hierro Majadahonda of Madrid, Spain (acta nº 03.17, dated 13 February 2017) and by the Bioethic Subcommittee- Ethics Commitee (CSIC) (dated 21 February 2017). Written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Availability of supporting data

The datasets during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

BOA and BBdM planning the study; Sample and material preparation, data collection and data analysis were performed by BOA, ERR, RES, BBdM and MSP. BOA drafted the manuscript and it was corrected and approved by all the authors.

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Table 1. Characteristics and lipid profile (mmol/L). Mean ± standard deviation and (median).

	Total sample (n= 92)	Women (n=68)	Men (n=24)
Age (y)	54.5 ± 5.8 (55)	54.6 ±5.9 (55)	54.1 ± 5.4 (54)
BMI (kg/m^2)	24.8 ± 3.1 (24.3)	24.6 ± 3.2 (24)	25.5 ± 2.9 (26)
Glucose (mmol/L)	4.48 ± 0.50 (4.44)	4.42 ± 0.44 (4.44)	4.65 ± 0.63 (4.83)
Cholesterol	5.33 ± 0.75 (5.25)	5.38 ± 0.76 (5.26)	5.20 ± 0.71 (5.24)
HDL-cholesterol	1.64 ± 0.37 (1.62)	1.72 ± 0.35 [a] (1.76)	1.41 ± 0.28 [a] (1.41)
LDL-cholesterol	3.22 ± 0.66 (3.18)	3.19 ± 0.69 (3.13)	3.31 ± 0.57 (3.28)
Triglycerides	1.00 ± 043 (0.88)	0.98 ± 0.44 (0.85)	1.06 ± 0.39 (1.01)
Cholesterol+TG (mmol/L)	6.33 ± 1.18 (6.13)	6.36 ± 1.2 (6.1)	6.26 ± 1.1 (6.25)
Diastolic pressure (mm Hg)	80 ± 1 (78,5)	79 ± 10.1 (77.5)	83.1 ± 9 (79.8)
Systolic pressure	125.8 ± 17.8 (122)	124 ± 17.8 (120)	131.4 ± 17.1 (125.5)

[a] Significant differences between sexes (p= 0,002 for all variables, except for HDL p= 0,000)

Table 2. Serum concentrations and dietary intake of lutein and zeaxanthin and, MPOD [mean ± standard deviation and (median)].

	Total sample (n=92)	Women (n=68)	Men (n=24)
Serum concentrations			
Lutein (µmol/L)	0.37 ± 0.12 (0.36)	0.39 ± 0.11 [a] (0.37)	0.33 ± 0.11 [a] (0.32)
Zeaxanthin (µmol/L)	0.10 ± 0.05 (0.08)	0.10 ± 0.04 (0.10)	0.11± 0.06 (0.12)
Lutein+zeaxanthin/cholesterol+TG (µmol/mmol)	0.09 ± 0.03 (0.09)	0.09 ± 0.03 (0.09)	0.08 ± 0.03 (0.08)
Dietary intake			
Lutein+zeaxanthin (µg/day)	1503 ± 1433 (1089)	1446 ± 1219 (1037)	1664 ± 1939 (1177)
Lutein+zeaxanthin / 1000 kcal	724 ± 677 (520)	741 ±626 (548)	677 ± 819 (459)
Fruit intake (g/day)	375 ± 281 (323)	366 ± 261 (336)	400 ± 334 (240)
Vegetable intake	342 ± 150 (342)	342 ± 143 (342)	344 ± 173 (348)
Fruit+vegetable intake	717 ± 339 (650)	707 ± 313 (654)	744 ± 410 (649)
Energy intake (kcal/d)	2136 ± 299 (2073)	1990 ± 167 [a] (1960)	2550 ± 176 [a] (2503)
Macular pigment optical density (density units) (n=184 eyes)	0.34 ± 0.13 (0.34)	0.35 ± 0.13 [a] (0.36)	0.31 ± 0.13 [a] (0.31)

[a] Significant differences between sexes for serum lutein (p= 0,019), serum lutein/LDL-cholesterol (p=0,021), for serum lutein/cholesterol+TG (p=0,016), for energy intake (p= 0,000) and for MPOD (p=0,034)

Table 3. Statistically significant correlations (Spearman's rho, (*p* value) between lutein, zeaxantin, lipids and major food sources for dietary intake and serum concentrations.

	Serum					Diet	
	Lutein	Zeax.	Lutein+Zeax.	Lutein+Zeax. /chol.+TG	Cholesterol	Lutein+Zeax.	Lutein+zeax./1000 kcal
Serum concentrations							
Cholesterol	0.213 (0.010)		0.178 (0.002)				
HDL-cholesterol	0.274 (0.001)	0.132 (0.024)	0.253 (0.000)				
Dietary intake							
Lutein+Zeax.	0.363 (0.000)		0.333 (0.001)	0.358 (0.000)			
Lutein+Zeax./1000 kcal	0.376 (0.000)		0.331 (0.000)	0.366 (0.000)			
Fruit	0.273 (0.001)	0.203 (0.014)	0.280 (0.001)	0.324 (0.000)	-0.216 (0.009)	0.353 (0.001)	0.395 (0.000)
Vegetable						0.481 (0.000)	0.504 (0.000)
Fruit+vegetable	0.281 (0.001)	0.195 (0.019)	0.281 (0.000)	0.306 (0.000)		0.482 (0.000)	0.525 (0.000)

Table 4. Contrast threshold at different degrees of visual angle, without and with glare

	Total sample		Women		Men	
Visual angle of the estimulus (º)	Without glare	With glare	Without glare	With glare	Without glare	With glare
6.3	0.019 ± 0.011 (0.014) [0.017 , 0.020]	0.020 ± 0.016 (0.014) [0.018 , 0.022]	0.020 ± 0.012 (0.014) [0.020 , 0.021]	0.021 ± 0.016 (0.014) [0.018 , 0.023]	0.016 ± 0.008 (0.014) [0.015 , 0.018]	0.016 ± 0.007 (0.014) [0.015 , 0.018]
4.0	0.026 ± 0.021 (0.020) [0.023 , 0.029]	0.024 ± 0.020 (0.020) [0.023 , 0.028]	0.029 ± 0.026 (0.020) [0.025 , 0.033]	0.029 ± 0.025 (0.020) [0.025 , 0.033]	0.019 ± 0.010 (0.014) [0.017 , 0.021]	0.019 ± 0.011 (0.014) [0.017 , 0.022]
2.5	0.037 ± 0.034 (0.030) [0.032 , 0.042]	0.042 ± 0.036 (0.030) [0.036 , 0.047]	0.041 ± 0.039 (0.030) [0.036 , 0.047]	0.046 ± 0.041 (0.030) [0.040 , 0.052]	0.029 ± 0.017 (0.030) [0.050 0.032]	0.033 ± 0.023 (0.030) [0.029 , 0.038]
1.6	0.067 ± 0.054 (0.040) [0.059 , 0.074]	0.078 ± 0.066 (0.060) [0.068 , 0.087]	0.072 ±0.060 (0.060) [0.063 , 0.080]	0.085 ± 0.076 (0.060) [0.074 , 0.096]	0.053 ± 0.040 (0.040) [0.045 , 0.045]	0.060 ± 0.039 (0.060) [0.053 , 0.068]
1.0	0.147 ± 0.100 (0.110) [0.133 , 0.162]	0.176 ± 0.114 (0.160) [0.159 , 0.195]	0.156 ± 0.112 (0.110) [0.140 , 0.172]	0.187 ± 0.127 (0.160) [0.169 , 0.206]	0.120 ± 0.084 (0.110) [0.103 , 0.137]	0.144 ± 0.095 (0.110) [0.125 , 0.163]
0.7	0.307 ± 0.150 (0.320) [0.285 , 0.329]	0.352 ± 0.140 (0.320) [0.332 , 0.372]	0.310 ± 0.159 (0.320) [0.287 , 0.333]	0.348 ± 0.150 (0.320) [0.327 , 0.370]	0.269 ± 0.150 (0.230) [0.239 , 0.299]	0.313 ± 0.155 (0.320) [0.282 , 0.344]

Values are expressed as means ± SD, (medians).

Table 5. Statistically significant correlations (Spearmn's rho, (pvalue) between lutein, zeaxanthin and major food sources for their intake in serum and diet with MPOD (two eyes/subject).

	MPOD
Lutein (serum)	0.226 (0.000)
Zeaxanthin (serum)	0.177 (0.003)
Lutein+zeaxanthin (serum)	0.229 (0.000)
Lutein+zeax./chol.+TG	0.165 (0.005)
Lutein+zeax./LDL	0.159 (0.007)
Lutein+zeax./HDL	0.149 (0.011)
Fruit intake	0.156 (0.008)
Vegetable intake	0.184 (0.002)
Fruit +vegetable intake	0.202 (0.001)
Contrast threshold –visual angle of the stimulus	
Without glare	
6.3	- 0.121 (0.039)
4.0	-0.160 (0.006)
2.5	-0.182 (0.002)
1.6	-0.205 (0.000)
1.0	-0.193 (0.001)
0.7	-0.201 (0.001)
With glare	
6.3	-0.135 (0.022)
4.0	
2.5	-0.155 (0.008)
1.6	-0.152 (0.009)
1.0	-0.160 (0.006)
0.7	-0.142 (0.015)

Table 6. Multivariate regression analysis of biochemical and dietary factors and sex data associated with MPOD.

	β (DE)	***p***	**95% CI**
Constant	0.706 (0.109)	0.000	0.492 , 0.919
Lutein +zeaxanthin (serum)	0.023 (0.004)	0.000	0.015 , 0.031
Lutein+zeaxanthin/HDL	-0.907 (0.214)	0.000	-1.328 , -0.485
HDL-cholesterol	-0.00 (0.002)	0.000	-0.009 , -0.003

CI: confidence interval.

Table 7. Association between contrast threshold, without and with glare, and MPOD, biochemical and dietary factors and sex.

		Without glare			With glare		
Visual angle of the estimulus (°)		β (DE)	*p*	95% CI	β (DE)	*p*	95% CI
6.3	Intercept	-3.801 (0.074)	0.000	-3.946 , -3.656	-3.740 (0.080)	0.000	-3.897 , -3.583
	Sex	-0.186 (0.061)	0.002	-0.306 , -0.066	-0.254 (0.067)	0.000	-0.386 , -0.122
	MPOD	-0.392 (0.202)	0.052	-0.788 , 0.003	-0.395 (0.218)	0.070	-0.823 , 0.032
4.0	Intercept	-3.379 (0.094)	0.000	-3.563 , -3.195	-3.415 (0.092)	0.000	-3.596 , -3.235
	Sex	-0.393 (0.078)	0.000	-0.547 , -0.240	-0.392 (0.078)	0.000	-0.545 , -0.238
	MPOD	-0.513 (0.260)	0.048	-1.022 , -0.005	-0.393 (0.252)	0.119	-0.888 , 0.101
2.5	Intercept	-2.955 (0.101)	0.000	-3.154 , -2.757	-2.866 (0.099)	0.000	-3.062 , -2.671
	Sex	-0.359 (0.085)	0.000	-0.526 , -0.193	-0.309 (0.084)	0.000	-0.475 , -0.144
	MPOD	-0.723 (0.279)	0.010	-1.270 , -0.175	-0.677 (0.273)	0.013	-1.212 , -0.143
1.6	Intercept	-2.332 (0.101)	0.000	-2.531 , -2.134	-2.177 (0.100)	0.000	-2.373 , -1.981
	Sex	-0.292 (0.085)	0.001	-0.459 , -0.126	-0.331 (0.085)	0.000	-0.499 , -0.164
	MPOD	-0.950 (0.279)	0.001	-1.496 , -0.404	-0.890 (0.276)	0.001	-1.430 , -0.350
1.0	Intercept	-1.556 (0.098)	0.000	-1.748 , -1.365	-1.444 (0.097)	0.000	-1.633 , -1.255
	Sex	-0.257 (0.082)	0.002	-0.417 , -0.096	-0.260 (0.081)	0.001	-0.419 , -0.102
	MPOD	-0.938 (0.270)	0.001	-1.467 , -0.410	-0.712 (0.264)	0.007	-1.230 , -0.194
0.7	Intercept	-0.955 (0.085)	0.000	-1.121 , -0.789	-0.926 (0.077)	0.000	-1.076 , -0.776
	Sex	-0.134 (0.070)	0.055	-0.272 , 0.003	-0.103 (0.062)	0.097	-0.225 , 0.019
	MPOD	-0.672 (0.231)	0.004	-1.125 , -0.219	-0.394 (0.208)	0.058	-0.801 , 0.013