Lutein and zeaxanthin supplied by red/orange foods and fruits are more closely associated with macular pigment optical density than those from green vegetables in Spanish subjects

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Abbreviations

F+V, fruits and vegetables; GLM, general linear model; HPLC, high performance liquid chromatography; L+Z, lutein plus zeaxanthin; MPOD, macular pigment optical density.
Abstract

Lutein and zeaxanthin (L+Z) accumulate in the retina. Although vegetables are major contributors to their intake, a stronger association between fruit and macular pigment optical density (MPOD) has been reported. We hypothesized that L+Z intake from fruits would have a stronger association with L+Z status markers (MPOD, serum concentrations) than intake from vegetables or eggs and, that those associations would also differ according to plant foods color. 108 subjects (57 men)(20-35, 45-65y) were enrolled in a cross-sectional study. L+Z intake from fruits, vegetables and eggs was determined using three 24h diet recalls and a country-specific carotenoid database. Vegetables were the major contributors (75%) to L+Z intake, followed by eggs (10%) and fruits (4%). Vegetables supplied 86% and 84% of the L+Z intake and fruits 3% and 16%. Green foods supplied 78% and 52% of L+Z respectively, followed by red/orange (9%, 38%) and white/yellow (14%, 9%). Factorial analysis showed associations in older subjects. The explained variance of the first two principal components was 54% considering L+Z intake from fruit, vegetables and eggs, and 55% considering L+Z intake from plant foods grouped by color. MPOD is related to L+Z intake from fruits (0.264, p=0.003) and is independent of that from vegetables and eggs. MPOD is related to L+Z intake from red/orange foods (0.320, p=0.000) and the serum concentrations to that from green foods (0.222, p=0.11). Although vegetables and green foods of plant origin are the major contributors to L+Z intake, red/orange foods and fruits have the strongest relationship to MPOD in study participants (45-65y).

Key words

Lutein; zeaxanthin; fruit and vegetables; dietary intake; macular pigment optical density.
1. Introduction

High fruit and vegetable (F+V) intake is considered to be an indicator of diet quality that is associated with a lower risk of several noncommunicable diseases [1]. Among the many components of this food group that could be responsible for said association, acting alone or in combination, are lutein and zeaxanthin [2]. These compounds are plant pigments that belong to the group of carotenoids, subgroup of the xanthophylls, and are provided by the diet, mainly through vegetable, fruit and egg consumption [3]. Both are transported in blood to the different tissues by lipoproteins [4] and are accumulated in the retina, where they, along with meso-zeaxanthin (that is believed to be obtained from the dietary lutein in the retina), constitute what it is known as macular pigment. They act by filtering blue light and as antioxidants that may protect macular pigment from oxidative damage induced by light and by the high rate of oxidative metabolism in the eye. Both, but primarily lutein, have been investigated in relation to eye health and disease [5]. There is increasing evidence suggesting that lutein and zeaxanthin may protect against age-related maculopathy, one of the major causes of blindness in the developed world [6].

Macular pigment density can be considered a marker of long-term dietary exposure [7, 8] and is often positively associated with dietary and serum lutein and zeaxanthin concentrations [9]. To our knowledge, despite the fact that there are a number of studies on dietary intake of lutein and zeaxanthin [10], on the relationship between their intake from the whole diet and their concentrations in serum [11, 12], and on their relationship to chronic diseases as well as to eye health/disease [5], there is no data on the specific contribution of fruits, vegetables and eggs to dietary lutein and zeaxanthin intake and nutritional status, or on their association with macular pigment optical density (MPOD). This association would be influenced to a large
extent by the differing bioavailabilities of carotenoids derived from different food matrices [4, 13]. On the other hand, an increasing number of national and international agencies tend to emphasize the importance of eating F+V of different colors [14] based on the understanding that different color could mean different phytochemicals, which would mean different health benefits [15-17].

In a recent study we assessed lutein and zeaxanthin status simultaneously using the three possible markers - total diet, serum and MPOD. Regarding the correlations between MPOD and dietary intake of lutein and zeaxanthin (in the total diet) and the consumption of fruits, vegetables and eggs, the highest correlation corresponded to that found between MPOD and F+V consumption in the total sample group and in the older, but not in the younger group. Moreover, there was a higher correlation of fruit intake than vegetable intake with serum lutein and zeaxanthin concentrations [9]. Thus, taking into account previously cited reports, the hypothesis of the present study is that the lutein and zeaxanthin intakes from fruits, vegetables and eggs show different degrees of association with their status markers (MPOD and blood concentrations) in subjects from two age groups and that intake from fruit could have the strongest association with the MPOD. In addition and because of the above mentioned importance of eating F+V of different colors [14 - 17] the foods of plant origin are grouped according to their colors (red/orange, white/yellow, green). Thus, the aim of this study is to assess the relationship between the intake of lutein and zeaxanthin from fruit, vegetables and eggs, and from plant foods, grouped according their color, and the lutein and zeaxanthin status markers (MPOD and their serum concentrations) in two age groups of subjects. The identification of the major food sources of lutein and zeaxanthin most closely associated with the status markers of these carotenoids (concentrations in serum and MPOD)
can be useful for public health decisions-making regarding prevention of certain chronic diseases.

2. Methods and materials

2.1. Participants and experimental design

108 volunteers (54 men), divided into two age groups (20-35y, 45-65y) (means ±SD: 25.6±3.2y and 52.4±5.2y, respectively) were enrolled in a cross-sectional study and underwent blood sampling, assessment of the MPOD and three 24 h diet recalls. Blood samples were collected after overnight fast (at least 8 hours) and at the same time as the first (out of three) 24h diet recalls. The recruitment and selection process, serum concentrations of lutein and zeaxanthin, the MPOD and the sample size calculation (108 subjects needed to obtain a 10% difference in the MPOD (0.04 du) with 85% power and alpha error of 0.05) are published elsewhere [9]. Briefly, the inclusion criteria were cholesterolemia of 140 to 235 mg/dl, body mass index above 20 and under 30 kg/m² and mixed diet (no avoidance of any food groups). The age groups were established because of their different dietary habits and risk of age-related macular disease. Volunteers were asked to report information on the following exclusion criteria: consumption of dietary supplements, surgery for myopia, cataracts or macular degeneration, use of drugs or beverages/foods to control cholesterol level (e.g. phytosterol or n-3 fatty acid-enriched) and chronic diseases that can affect carotenoid or lipid metabolism. The participants were enrolled over the course of an entire year (during the spring and summer: 40 in the younger and 29 in the older age group; and, during the fall and winter: 14 in the younger and 25 in the older age group).
This study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human volunteers 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 subjects.

2.2. Dietary intake assessment

Recent dietary intake was evaluated using three 24h diet 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 (for fruits), portions or household servings [3]. 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 carotenoid database developed by our group, included in a software application for the calculation of dietary intake of individual carotenoids [18, 19]. This carotenoid database contains data on the major dietary carotenoids present in foods, and all of them were generated by high-performance liquid chromatography (HPLC) [19-21] using an analytical procedure that was considered as highly acceptable [22]. The foods included, mainly from the plant kingdom, are major contributors to the intake of carotenoids in Europe [23, 24]. The food groups included in the software are: fruit, vegetables, oils and fats, snacks, nonalcoholic beverages, milk and dairy products, eggs and egg products, and sauces and seasonings. The individual lutein and zeaxanthin content of each of these items was multiplied by the amount consumed (g/person/day) and this provided an estimate of the intake of these xanthophylls in the whole diet, that was previously
published [9] and is included in table 1 for comparative purposes. In the present study, we focus on the amount of lutein and zeaxanthin supplied by fruits, vegetables and eggs, breaking the F+V group down according to color (red/orange, white/yellow, green).

The carotenoid database used [19] includes data on lutein and zeaxanthin in 124 foods, expressed separately in all but 27 foods for which the literature provides data only on the combined lutein and zeaxanthin (L+Z) content (e.g. eggs and certain foods that were not frequently consumed by the participants in this study). Thus, the dietary intake of lutein and zeaxanthin is expressed both separately and as the sum of the two, and, consequently, in some cases, the value for L+Z does not coincide with the sum of the concentration of each. Of these 27 foods for which lutein and zeaxanthin are expressed as their sum, only 19 were registered in the dietary records by one or more volunteers.

There was no difference in the energy intake between the two age groups, and the difference found in lutein and zeaxanthin intake (which was higher in the older group) was maintained when intake was expressed in terms of dietary energy density (L+Z / 1000 kcal) [9]. As lutein and zeaxanthin in serum showed a high correlation with MPOD when these xanthophylls were expressed in relation to serum lipids (in older participants) [9], we evaluated the lipid intake using a food composition table widely accepted in Spain [3]. The intake of total lipids and lipids divided according to the different families of fatty acids (monounsaturated fatty acids [MUFA], polyunsaturated fatty acids [PUFA] and saturated fatty acids [SFA]) are expressed in g/day.

2.3. Lutein and zeaxanthin analysis in blood
Lutein and zeaxanthin concentrations were determined by HPLC and the procedure and results have been published elsewhere [9]. Briefly, the system used consisted of a model 600 pump, a Rheodyne injector and a 2998 photodiode array detector (Waters, Milford, MA, USA); a Spheri-5 ODS 5 μm (220 mm×4.6 mm) chromatographic column (Brownlee Labs, Applied Biosystem, Santa Clara, CA, USA) with a guard column (Aquapore ODS type RP-18) were used. The mobile phase changed from acetonitrile-methanol (85:15; v/v), to acetonitrile-dichloromethane-methanol (70:20:10; v/v/v) in a linear gradient from min 5 to min 20 at a flow rate of 1.8 mL min\(^{-1}\). Detection was performed at a wavelength of 450 nm and chromatograms were processed using Empower 2 software (Waters, Milford, MA, USA). Carotenoid extraction was performed on serum samples as described in a previously published method [25].

2.4. Macular pigment optical density 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. [26]. 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 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 2º diameter target placed 8º eccentrically and a second set of data were recorded for peripheral viewing [27]. The MPOD was measured in density units (du) and ranges from 0 to 1. The procedure and results have been published elsewhere [9].
2.5. Statistical analyses

Data are expressed as the means and standard deviation and median. To detect possible intergroup differences in the variables “age” and “sex”, the mean values were compared using parametric and nonparametric tests (Student t-tests, Mann Whitney-U test), given that it was difficult to confirm the normal distribution in some of the variables analyzed. In this way, we were able to confirm that the differences observed were not a consequence of a skewed distribution of the variables. For the same reason, to detect possible differences between the lutein and zeaxanthin intake derived from the food groups, a parametric test (paired t-test) and a nonparametric test (Wilcoxon) were performed.

We analyzed the possible differences in the dependent variable “MPOD”, taking into account the factors “age” and “sex”. General linear model (GLM) analysis revealed that age had a significant effect on MPOD, and that there was an interaction between age and sex in the older age group.

The analysis of the relationships between the diet and serum variables was carried out using a Pearson correlation matrix. The analysis of the relationships between the main outcome measure “MPOD” and the intake variables was performed jointly by means of factor and GLM analysis (including sex, age and their interaction).

The factor analyses were performed, on the one hand, with dietary lutein and zeaxanthin intake from fruits, vegetables and eggs, and, on the other hand, with those intakes from foods classified according color. To assess the intakes from fruits, vegetables and eggs, and because
the concentration of lutein and zeaxanthin in eggs is expressed jointly, the data on lutein and zeaxanthin introduced into the factor analysis was the L+Z data. As eggs were not found to be associated with MPOD in the factor and GLM analyses, and since for the remainder of the foods there are separate data for lutein and zeaxanthin, we undertook the analysis of the individual intakes of lutein and zeaxanthin from fruit and vegetables.

Two factorial analyses were performed (extraction method: principal component analysis; rotation method: Varimax rotation with Kaiser normalization) to examine the following sets of variables: MPOD, the serum concentrations of lutein, zeaxanthin and lutein plus zeaxanthin and, 1) the lutein and zeaxanthin intake from fruit, vegetables and eggs (as major contributors to the dietary intake of these carotenoids), 2) the lutein and zeaxanthin intake from fruit and vegetables grouped by color red/orange, white/yellow, green). Factor analysis was carried out for each of the two age groups. The objective is to identify multivariate relationships taking each correlation matrix. The results are presented in the form of plots of the component loadings. Each point is connected to the origin and the angles between segment express the measurement of the correlation (angles narrower than 90º indicate positive correlation, wider angles indicate negative correlation).

All reported P-values were compared to a significance level of 5%. SPSS v.21 (SPSS Inc., Chicago, IL, USA) software was used for all statistical calculations.

3. Results

3.1. Contribution of fruits, vegetables and eggs to dietary lutein and zeaxanthin intake
Table 1 shows the intakes of lutein and zeaxanthin from fruits, vegetables and eggs by the group as a whole and according to sex and age. In addition, the table includes published data on the intake of lutein and zeaxanthin from the whole diet, on dietary fruit, vegetable and egg intake (grams) and on MPOD [9] for a better comprehension of the study.

The consumption of F+V was similar in men and women, but was higher in the older age group compared to that of the younger volunteers. In contrast, there were no differences between the sexes or age groups in terms of egg consumption. Of the foods in the study, those most widely consumed were vegetables, followed by fruits and eggs [9].

To determine whether the greater contribution of lutein and zeaxanthin from vegetables was due to a higher consumption of these foods (g) or to vegetables being richer in lutein and zeaxanthin than fruits and eggs, we calculated the amount of L+Z per gram of food supplied by each of those food groups. Eggs were the food group that provided the most L+Z per gram (median, 5.46 μg/g), supplying nearly 4 times more than vegetables (median, 1.33 μg/g) and around 32 times more than fruits (median, 0.17 μg/g). Vegetables provided approximately 8 times more L+Z than fruits.

There were significant differences among the contributions of fruits, vegetables and eggs to the dietary intake of lutein, zeaxanthin and L+Z. In the overall sample, vegetables supplied 86% and 84% of the lutein and zeaxanthin intake, respectively. Fruits supplied only 3% and 16% of lutein and zeaxanthin, respectively. When L+Z intake was considered, vegetables were the major contributors (75%), followed by eggs (10%) and fruits (4%). Both xanthophylls are also supplied by other foods (11%), such as ketchup (group of sauces and seasonings) and orange and tomato juices (nonalcoholic beverage group).
The intake of lutein and zeaxanthin from the three food groups by men and women was similar for practically all the study variables. In contrast, there were a number of differences between the two age ranges, with higher amounts being ingested by the older group.

3.2. Lutein and zeaxanthin intake according to the color of foods of plant origin

Table 2 shows the lutein and zeaxanthin intake (µg/day) from food groups analyzed according to the colors red/orange, white/yellow and green. There were significant differences in lutein, zeaxanthin and L+Z intake from the three color groups, except for the intake of zeaxanthin when red/orange and green foods were compared.

The foods of plant origin that supplied the highest amount of lutein (78%) and zeaxanthin (52%) were those of green color, followed by red/orange (9% of lutein and 38% of zeaxanthin intake) and white/yellow (14% of lutein and 9% of zeaxanthin intake). Regarding L+Z, the major contributors were foods of green color (64%); white/yellow and red/orange items supplied similar amounts (14% and 12%, respectively). Other foods that contributed with 10% were eggs and egg yolk, but were not considered in the calculation of the above percentages as they are not of plant origin.

As was the case with the intake of lutein and zeaxanthin from fruits, vegetables and eggs, there was little difference between men and women when the food sources were grouped according to their colors, although there were differences between the age groups (higher in the older group).
Of the F+V classified in the red/orange group, those that were consumed by the largest number of individuals in the overall study group were: tomato (raw) (84%), carrot (raw and cooked) (60%), tomato (saucess) (46%), orange juice (48%) (25% from concentrates and 23% from natural juice), watermelon (22%) and peach (21%). Of the group of foods classified by color as white/yellow, the most widely consumed item was olive oil (99%), followed by onion (86%), potato (85%), banana and apple (36% each) and zucchini (25%). Finally, in the group of green produce, the leading items were green peppers (63%), lettuce (61%) and, to a much lesser extent, green beans (29%), peas (19%) and spinach (19%).

3.3. Correlations between lutein and zeaxanthin intake from fruits, vegetables and eggs and their serum concentrations

Table 3 shows the low significant correlations between the amounts of these carotenoids provided by fruits, vegetables and eggs, as well as those derived from the foods grouped according to color and the serum concentrations of lutein, zeaxanthin and L+Z in the total study group and in the two age groups. In the total study group, lutein and zeaxanthin intakes from eggs exhibited no correlation with their concentrations in serum and, the variables that showed the highest number of significant, but low, correlations were the lutein and zeaxanthin intakes from fruits and from red/orange foods. However, considering age, in the younger group, significant correlation was found only between L+Z intake and their serum concentrations, and it was higher than that in the total study group (0.474 vs 0.264).

3.4. Association of MPOD with lutein and zeaxanthin intake and serum concentrations
To determine which of the three food groups studied had the greatest association with MPOD and with the lutein and zeaxanthin serum concentrations, we performed factor analysis in which the intake of L+Z from each of them was introduced (because there were no individual data for lutein and zeaxanthin in eggs). In the total study group, the univariate GLM analysis of the L+Z intake from each of the three food groups ruled out a significant association with the intake of L+Z from eggs and vegetables on MPOD. The intake derived from fruits did have an association (p=0.007), explaining only 9.2% of the variance in MPOD. Sex, age and their interaction were included in all of the GLM analyses because of age-related differences (less marked among older vs younger individuals; p=0.038). In the older age group, sex was a predictor of MPOD (men had higher MPOD than women), as we reported elsewhere [9]. The GLM analysis showed the association of lutein and zeaxanthin intake from foods in the red/orange group (p=0.000 and p=0.020, respectively) with the MPOD was shown, with lutein intake explaining 12.7% of the variance in MPOD and zeaxanthin intake, 8.4%. However, lutein and zeaxanthin derived from green and white/yellow foods had no significant effects in those models in which they were included, despite the fact that the vegetables and foods of green color were the major contributors to lutein and zeaxanthin intake. For this reason, and because the database includes a high proportion of vegetables among the foods classified as green (32 of 42), we used factor analysis to study whether the intake of lutein and zeaxanthin from F+V was associated with the intake of lutein and zeaxanthin from foods classified according to color.

The factorial analysis of the variables MPOD, L+Z intake supplied by fruits, vegetables and eggs, L+Z intake supplied by plant foods grouped according to their color (red/orange, white/yellow, green) and L+Z serum concentration was performed in the two age groups. In the younger subjects, the variance showed independence among them. In contrast, in the older
In the older group, there were statistically significant associations between the L+Z intake and their status markers (MPOD and serum concentrations), the degree of explained variance of the first two principal components being 54% in the first case, in which L+Z intake from fruit, vegetable and eggs was considered, and 55% in the second case, in which their intake from plant foods grouped according to their color was considered. The correlation matrix for MPOD, L+Z serum concentrations and L+Z intake from fruits, vegetables and eggs, in the older group is shown in figure 1. MPOD is related to L+Z intake from fruits (0.264, \( p = 0.003 \)) and is independent of the L+Z intake from vegetable and eggs; the L+Z serum concentrations is associated with L+Z intake from vegetables (0.215, \( p = 0.013 \)). Figure 2 represents the associations among variables considering the L+Z intakes from plant foods grouped according to their color in the older group. The two status markers show different associations with the lutein and zeaxanthin intake from the food sources that supply them. While MPOD is closely related to the L+Z intake from red/orange foods (0.320, \( p = 0.000 \)) and related to the intake from white/yellowish foods (0.217, \( p = 0.12 \)), the serum status marker is associated with the L+Z intake from green foods (0.222, \( p = 0.11 \)). The association between L+Z status markers is 0.249 (\( p = 0.005 \)). Although in the figures corresponding to the factor analysis, we have used data from the sum of lutein and zeaxanthin, the separate components (lutein, zeaxanthin and L+Z) show the same behavior.

Finally, we calculated total fat intake to determine whether it was related to MPOD. Intake of lipids (g) and fatty acid families (g) for the total sample, expressed as means ±SD, [95% CI] and (median), were: lipids, 90.5±30.6 [86.4; 94.6] (87.3); SFA, 27.9±11.2 [26.4; 29.4] (25.7); MUFA, 38.7±14.4 [36.8; 40.6] (36.5); and PUFA, 15.4±7.3 [14.5; 16.4] (13.9). Factor analysis showed that the intake of lipids, either total or classified by family, was not associated with the MPOD.
4. Discussion

The present study focuses on the lutein and zeaxanthin dietary intakes from fruits, vegetables and eggs and their associations with their status markers (MPOD and blood concentration). To our knowledge, there are no studies with information on the specific amounts of these carotenoids provided individually by fruits, by vegetables and by eggs, although we do have data on intake from the total diet [10]. However, we consider that knowledge of the individualized sources can facilitate nutritional intervention programs [11, 28, 29] in public health designed to favor an increase in the MPOD in certain population groups. Nonetheless, F+V present a broad range of colors, each of which is associated with different phytochemical profiles and, thus, with different biological activities [29]. The carotenoids are also associated with certain colors: lycopene with red-colored foods, provitamin carotenoids with orange-colored foods and lutein and zeaxanthin with dark-green-colored foods [30]. The classification of F+V according to the color of their edible portion is becoming increasingly common in recent years, especially for the establishment of public health dietary recommendations. 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 fact that the study was designed with the main objective of assessing differences in the dietary and status markers of study participants in two age ranges; thus, the sample size was calculated on the basis of the MPOD value [detailed in ref. 9]. Based on the findings, the hypothesis of this study can be accepted, as the associations between L+Z from fruit and vegetables and MPOD and serum concentrations differ in the older group of subjects and no association was found with egg intake. Moreover, the
red/orange plant foods (mainly fruits) are more closely associated with the MPOD than the green plant foods.

4.1. Lutein and zeaxanthin supplied by fruits, vegetables and eggs

In the present study, vegetables constitute the food group that supplies the highest amounts of lutein and zeaxanthin to the diet. This greater contribution is not due only to the fact that these foods are consumed in the largest quantities (g), but also to the fact that the amount of L+Z per gram is much higher than that in fruits. However, it is not greater than the amount in eggs, in which it is four-fold higher, but egg consumption (g) in the total study group is 10 times lower than that of vegetables and, thus, their contribution to the total intake is smaller.

Our findings agree with the recommendation to increase the consumption of green-colored foods in order to increase the intake of lutein and zeaxanthin [14], as it was those foods that provided the highest percentage of lutein and zeaxanthin to the diet of our volunteers, and with the results of Moeller et al. [31].

The database on carotenoid content utilized in this study [19] to determine the intakes of foods of plant origin, classified according to color, takes into account only the color of the edible portion, as occurs in other studies [15-16]. However, the classification of foods according to color does not follow the same pattern in all the studies; for instance, apple is classified as a white fruit in this study and as yellowish in Mirmiran [15]; the USDA food pattern classifies green beans and avocados as “other vegetables”, but in the present study they are classified as green vegetables. With the exception of this aspect, and the fact that the data in this study are expressed as percentage of consumers, whereas data from other studies
represent the consumption of each food in grams, expressed as a percentage of the overall consumption in grams of all the foods in its color group, it could be said that many of the most widely consumed foods in the different studies coincide. This shows that, as previously described [24], a small number of foods constitute the major contributors to carotenoid intake in population groups.

4.2. Correlations of lutein and zeaxanthin supplied by fruits, vegetables and eggs with their concentrations in serum

The plasma carotenoid concentration is considered a marker of fruit and vegetable intake [32]. Specifically, serum lutein and zeaxanthin concentrations were much more strongly associated with fruit intake (Spearman coefficient=0.318) than with vegetable intake (Spearman coefficient=0.255) in a previous study involving the same participants included in the present study [9]. In order to examine this aspect in greater depth, we assessed the relationship between serum lutein and zeaxanthin concentrations and their intake from fruits, vegetables and eggs. We observe that there are a greater number of correlations and with higher coefficients for lutein and zeaxanthin intake from fruits than from vegetables in the total study group and in the younger group, and the opposite in the older group, in spite of the fact that older subjects in this study consumed nearly twice as much fruit (median: 269 vs 152 g/d) and somewhat greater amounts of vegetables (291 vs 231 g/d) than the younger subjects [9]. However, there are studies reporting a higher correlation coefficient for vegetable intake (g) (Pearson coefficient=0.43) than for fruit intake (Pearson coefficient=0.21) [32]. Aside from the amount consumed, the degrees of correlation between biological markers are influenced by many other food and subject-related factors (e.g. eating habits, dietary interactions), as well as metabolic factors [8] that may influence metabolism of these xanthophylls, such as the
uptake of lutein and zeaxathin from plasma and their transport to the retina (e.g. serum lipid levels or binding protein in optical tissue) [12].

No significant correlation is observed between lutein and zeaxanthin supplied by eggs and the serum concentrations of these xanthophylls; nor is there any correlation between egg intake (grams) and serum lutein and zeaxanthin concentrations [9]. Considering the high bioavailability of lutein and zeaxanthin from egg [33], it is difficult to understand the lack of correlation found in this study, although perhaps it can be explained in part by the lower egg consumption (25 g) compared to the mean intake in the Spanish population (32 g) [34] or by a low lutein and zeaxanthin content in the eggs consumed [35].

As is widely known, lutein and zeaxanthin are associated with dark green foods. However, the red/orange food group has shown numerous correlations and the highest coefficients of the three groups, and lutein from green foods exhibited no significant correlation with serum concentrations. A lack of correlation between foods of green color (frequency of consumption) and serum lutein and zeaxanthin concentrations was reported [36].

In general, the correlation coefficients between the intake of lutein and zeaxanthin and their concentrations in serum are low in all the studies in the literature [37], a finding that might be explained by the wide variability in the carotenoid content in foods [38] by the manner of expressing the data in food composition tables [39] by the fact that the dietary assessment does not reflect the amount consumed and absorbed or by the variability in inter-individual responses [11, 40].
4.3. Relationship between lutein and zeaxanthin intake from fruits, vegetables and eggs and MPOD

The consumption of fruits, vegetables and eggs, major contributors to lutein and zeaxanthin intake, has been associated with MPOD [11, 31, 41], as has the lutein and zeaxanthin intake from the whole diet [10, 11, 31, 41]. As we reported elsewhere, the correlation between MPOD and fruits (grams) was higher than that with vegetables (in the older group), and there was no correlation with eggs [9]. When dietary lutein and zeaxanthin intake from major contributors is assessed, we observe that the correlation between the amounts provided by fruits (µg/day) and MPOD is higher than that found with vegetables, and that there is no correlation with egg intake. On the other hand, in an epidemiological study assessing MPOD and fruit and vegetable intake, a lower risk of age-related macular degeneration was associated with a higher intake of fruit but not of vegetables [42].

The fact that the correlation between MPOD and lutein and zeaxanthin intake from fruits is higher than those from vegetables and from eggs, although F+V both supply high amounts of lutein and zeaxanthin and eggs contain higher concentrations of these two xanthophylls, could be explained by a higher bioavailability of lutein and zeaxanthin from fruits. These xanthophylls are found in the ester forms in fruits [40], which could have a greater bioavailability than free forms [43], although this explanation is not widely accepted [40]. On the other hand, although the relationship between bioaccessibility or bioavailability of carotenoids and the different types of chromoplasts in which they are found is not clearly established [44], the carotenoids in fruits are found mainly in chromoplasts and are more efficiently released by digestion than carotenoids in green vegetables, which are mainly
located in chloroplasts [13, 44, 45]. In this respect, the influence of possible interactions among carotenoids cannot be ruled out [46, 47].

Considering F+V according to colors, those of green color were the foods that contributed the greatest amount of lutein and zeaxanthin to the diet of our volunteers. However, the red/orange foods are those that have the greatest correlation on MPOD, and this color group is closely related to fruit consumption.

As fat intake favors carotenoid bioaccessibility [48] and MPOD in the participants in this study showed age-specific correlations with L+Z expressed in relation to serum lipid concentrations [9], the lutein and zeaxanthin intake levels were studied in terms of the lipid intake (total lipids, saturated, MUFA and PUFA) to assess their relationship to MPOD. Although, there are significant positive correlations (Pearson coefficients), the association of those intakes on MPOD were ruled out by means of factor analysis. This lack of a relationship to dietary lipid intake has been reported elsewhere [31] but not in another study in which PUFA intake was assessed [11].

Finally, we can conclude that the contribution of vegetables to the dietary intake of lutein and zeaxanthin is more than seven times greater than that of eggs and nearly twenty-fold that of fruits. Regarding food colors, major contributors to lutein and zeaxanthin intake are green plant foods, followed by red/orange and white/yellow foods. However, the lutein and zeaxanthin supplied by red/orange foods and the group of fruits (most of the fruits are red/orange or white/yellow in color), have the greatest association with MPOD in study participants aged 45-65y.
Acknowledgements

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References


Figure 1. Factor analysis loading plot. Two principal components. Older study group (45-65 y).

Sum_lut_zeax_veg: lutein + zeaxanthin intake supplied by vegetables; Sum_lut_zeax_fruit: lutein + zeaxanthin intake supplied by fruits; Sum_lut_zeax_egg: lutein + zeaxanthin intake supplied by eggs; MPOD: macular pigment optical density; lut_zeax_serum: lutein + zeaxanthin serum concentration.
Figure 2. Factor analysis loading plot. Two principal components. Older study group (45-65y).

Sum_lut_zeax_GR: lutein + zeaxanthin intake supplied by green plant foods;
Sum_lut_zeax_WY: lutein + zeaxanthin intake supplied by white/yellow plant foods;
Sum_lut_zeax_RO: lutein + zeaxanthin intake supplied by red/orange plant foods; MPOD: macular pigment optical density; lut_zeax_serum: lutein + zeaxanthin serum concentration.
Table 1. Dietary intake of lutein, zeaxanthin and lutein+zeaxanthin from fruits, vegetables and eggs, intake of foods and MPOD*

<table>
<thead>
<tr>
<th>Dietary intake</th>
<th>Total sample</th>
<th>Men 20-35 years</th>
<th>45-65 years</th>
<th>Women</th>
<th>20-35 years</th>
<th>45-65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SD</td>
<td>Median</td>
<td>Means ± SD</td>
<td>Median</td>
<td>Means ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/day†</td>
<td>224.4 ± 186.9</td>
<td>197.5</td>
<td>225.4 ± 196.5</td>
<td>200.0</td>
<td>223.4 ± 177.7</td>
<td>193.9</td>
</tr>
<tr>
<td>L</td>
<td>37.4 ± 38.3</td>
<td>22.5</td>
<td>31.9a ± 39.4</td>
<td>15.2</td>
<td>43.0a ± 36.5</td>
<td>39.0</td>
</tr>
<tr>
<td>Z</td>
<td>15.5 ± 28.3</td>
<td>0.0</td>
<td>13.9 ± 29.2</td>
<td>0.0</td>
<td>17.0 ± 27.5</td>
<td>0.0</td>
</tr>
<tr>
<td>L+Z</td>
<td>53.4 ± 59.7</td>
<td>34.1</td>
<td>46.7a ± 64.7</td>
<td>17.2</td>
<td>60.2a ± 53.8</td>
<td>51.2</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/day†</td>
<td>274.9 ± 148.4</td>
<td>266.3</td>
<td>280.8 ± 157.3</td>
<td>261.2</td>
<td>269.1 ± 139.4</td>
<td>271.4</td>
</tr>
<tr>
<td>L</td>
<td>921.5 ± 1567.7</td>
<td>248.1</td>
<td>815.4 ± 1415.0</td>
<td>256.4</td>
<td>1027.7 ± 1706.9</td>
<td>191.8</td>
</tr>
<tr>
<td>Z</td>
<td>79.6 ± 139.0</td>
<td>22.0</td>
<td>64.6 ± 119.0</td>
<td>19.7</td>
<td>94.6 ± 155.6</td>
<td>25.3</td>
</tr>
<tr>
<td>L+Z</td>
<td>1080.4 ± 1727.9</td>
<td>317.9</td>
<td>996.8 ± 1610.5</td>
<td>305.3</td>
<td>1164.1 ± 1841.5</td>
<td>330.7</td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/day†</td>
<td>25.3 ± 25.2</td>
<td>21.3</td>
<td>25.9 ± 28.4</td>
<td>21.3</td>
<td>24.8 ± 21.7</td>
<td>21.3</td>
</tr>
<tr>
<td>L+Z</td>
<td>143.2 ± 139.8</td>
<td>116.4</td>
<td>149.8 ± 157.6</td>
<td>116.4</td>
<td>136.7 ± 119.8</td>
<td>116.4</td>
</tr>
<tr>
<td>Total diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L†</td>
<td>1073.1 ± 1573.7</td>
<td>410.5</td>
<td>955.4 ± 1411.5</td>
<td>408.8</td>
<td>1190.8 ± 1719.3</td>
<td>410.5</td>
</tr>
<tr>
<td>Z†</td>
<td>95.1 ± 139.5</td>
<td>38.1</td>
<td>78.6 ± 120.6</td>
<td>31.8</td>
<td>111.6 ± 155.0</td>
<td>48.9</td>
</tr>
<tr>
<td>L+Z†</td>
<td>1449.4 ± 1742.0</td>
<td>745.1</td>
<td>1380.2 ± 1641.4</td>
<td>736.9</td>
<td>1518.6 ± 1842.2</td>
<td>761.2</td>
</tr>
<tr>
<td>MPOD†</td>
<td>0.35 ± 0.16</td>
<td>0.36</td>
<td>0.35 ± 0.16</td>
<td>0.36</td>
<td>0.34 ± 0.16</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Dietary intake is expressed as (μg/day), intake of foods is expressed as g/day.

* There are significant differences among the contributions of fruits, vegetables and eggs.

L, Lutein; Z, zeaxanthin; L+Z, Lutein+zeaxanthin; MPOD, macular pigment optical density.

Mann- Whitney U test. a Significant differences between sexes. b Significant differences between age groups.

† From Olmedilla et al. 2014 9
Total sample (n=108); men (n=54); women (n=54); 20-35 years (n=54); 45-65 years (n=54)
Table 2. Dietary intake of lutein, zeaxanthin and lutein+zeaxanthin from foods classified by colour*

<table>
<thead>
<tr>
<th>Dietary intake</th>
<th>Total study group</th>
<th>Men</th>
<th>Women</th>
<th>20-35 years</th>
<th>45-65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SD</td>
<td>Median</td>
<td>Means ± SD</td>
<td>Median</td>
<td>Means ± SD</td>
</tr>
<tr>
<td>White/yellow</td>
<td>L</td>
<td>147.5 ± 58.2</td>
<td>139.8</td>
<td>142.5 ± 56.5</td>
<td>134.1</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>9.4 ± 9.4</td>
<td>6.3</td>
<td>10.6a ± 8.4</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>L+Z</td>
<td>199.3 ± 149.9</td>
<td>161.6</td>
<td>196.5 ± 164.4</td>
<td>161.6</td>
</tr>
<tr>
<td>Red/orange</td>
<td>L</td>
<td>94.0 ± 66.1</td>
<td>87.8</td>
<td>98.0 ± 72.1</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>36.4 ± 55.9</td>
<td>14.9</td>
<td>23.4a ± 34.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>L+Z</td>
<td>172.6 ± 137.1</td>
<td>152.2</td>
<td>183.9 ± 158.5</td>
<td>148.1</td>
</tr>
<tr>
<td>Green</td>
<td>L</td>
<td>835.5 ± 1563.9</td>
<td>146.6</td>
<td>721.7 ± 1411.5</td>
<td>166.5</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>49.3 ± 129.5</td>
<td>0.0</td>
<td>44.6 ± 118.9</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>L+Z</td>
<td>933.4 ± 1706.8</td>
<td>164.7</td>
<td>848.2 ± 1583.0</td>
<td>186.9</td>
</tr>
</tbody>
</table>

Dietary intake is expressed as (μg/day).

* There are significant differences among contributions from white/yellow, red/orange and green foods, except between zeaxanthin intake from red/orange and green foods.

L, Lutein; Z, zeaxanthin; L+Z, Lutein+zeaxanthin

Mann-Whitney U test. a Significant differences between sexes and b Significant differences between age groups.

Total sample (n=108); men (n=54); women (n=54); 20-35 years (n=54); 45-65 years (n=54)
Table 3. Statistically significant correlations between serum concentrations of lutein and zeaxanthin and dietary intake of those carotenoids according to food source $^a$.

<table>
<thead>
<tr>
<th>Dietary intake</th>
<th>Serum concentration</th>
<th>Total study group</th>
<th>20-35 years</th>
<th>45-65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>Z</td>
<td>L+Z</td>
<td>L</td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>0.172 (0.011)</td>
<td>0.149 (0.029)</td>
<td>0.195 (0.043)</td>
<td>0.194 (0.044)</td>
</tr>
<tr>
<td>L+Z</td>
<td>0.185 (0.006)</td>
<td>0.174 (0.010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.176 (0.01)</td>
<td>0.177 (0.009)</td>
<td>0.159 (0.020)</td>
<td>0.176 (0.01)</td>
</tr>
<tr>
<td>Z</td>
<td>0.180 (0.008)</td>
<td>0.242 (0.012)</td>
<td>0.220 (0.022)</td>
<td>0.240 (0.012)</td>
</tr>
<tr>
<td>L+Z</td>
<td>0.173 (0.011)</td>
<td>0.200 (0.003)</td>
<td>0.170 (0.012)</td>
<td>0.229 (0.017)</td>
</tr>
<tr>
<td>White/ yellow foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0.218 (0.001)</td>
<td>0.160 (0.019)</td>
<td>0.203 (0.003)</td>
<td>0.326 (0.001)</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L+Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/ orange foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>0.146 (0.032)</td>
<td>0.275 (0.000)</td>
<td>0.206 (0.002)</td>
<td>0.410 (0.000)</td>
</tr>
<tr>
<td>L+Z</td>
<td>0.204 (0.003)</td>
<td>0.286 (0.000)</td>
<td>0.229 (0.001)</td>
<td>0.451 (0.000)</td>
</tr>
<tr>
<td>Green foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L+Z</td>
<td>0.171 (0.012)</td>
<td>0.166 (0.014)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pearson correlation coefficients and ($p$-value) (n=108)

$^a$ L and Z intake from eggs showed no significant correlations with L and Z concentrations in serum
L=Lutein, Z=Zeaxanthin, L+Z=Lutein+zeaxanthin
Significant differences in serum L concentrations were found between the 45-65 y group (13.03±6.61; 5.22-37.46; 14.76) and the 20-35 y group (9.90±5.02; 3.38-7.46; 10.90) but not in serum Z concentrations [9]
Highlights

Correlations of lutein and zeaxanthin intake (fruit, vegetables) with their status markers

Lutein and zeaxanthin from fruits but not from vegetables are associated with MPOD.

Lutein and zeaxanthin intake from red/orange plant foods have the greatest association with MPOD

Lutein and zeaxanthin intake from green foods is associated with their serum levels