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ORIGINAL CONTRIBUTION

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Development and validation of a brief food frequency questionnaire for dietary lutein and zeaxanthin intake assessment in Italian women

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Abstract *Background* There is increasing evidence that higher intakes of carotenoids could protect against oxidative and light damage in premature infants and may promote other health benefits in both mothers during pregnancy and lactation and in newborn infants. *Aim of the study* To develop and validate a brief quantitative food frequency questionnaire (FFQ) aimed at assessing lutein and zeaxanthin intake in women. *Methods* In this cross-sectional study, estimates of lutein and zeaxanthin intake from the FFQ were compared with a 7-day dietary record and with plasma concentrations of these carotenoids. This primary care study was conducted in Pavia, Italy. Subjects were all female volunteers, aged 20–25 years (mean age 22.7 ± 2.1 years), university students. Of the 110 women initially recruited, 87 completed diet questionnaires and donated a blood sample. Dietary intake was assessed by the FFQ by interview and 7-day dietary records chosen as a reference standard, using photographic estimations of portion sizes. Plasma concentrations of lutein and zeaxanthin were measured by HPLC. Pearson's correlation coefficient and Bland Altman Regression analysis were used. *Results* Mean dietary lutein and zeaxanthin intakes were 1,107 ± 113 μg/day from the FFQ questionnaire and 1,083 ± 116 μg/day from the 7 day dietary records. The mean difference in intake assessed by the two methods (−24.5 ± 38.3 μg/day) did not differ significantly from zero. Dietary intake of lutein and zeaxanthin measured with the FFQ and plasma nutrient concentration among this sample were significantly correlated ($r = 0.76$, $P < 0.0001$). Mean plasma lutein and zeaxanthin concentrations were 0.33 ± 0.09 μmol/l. *Conclusions* This FFQ could be used to assess lutein and zeaxanthin intake in adult women.

Key words FFQ – lutein (L) and zeaxanthin (Z) intake – plasma lutein (L) – zeaxanthin (Z)

Introduction

There is increasing evidence that higher intakes of carotenoids are associated with reduced risk for a variety of chronic diseases, including cardiovascular disease, age related macular degenerations and some cancers [10, 11, 31], as well as protection against oxidative and light damage in premature infants [20], and

other health benefits in both mothers during pregnancy and lactation, and in new born infants [16].

Several studies have compared various measurements for dietary intake of carotenoids with plasma concentrations [3, 29, 37, 39, 45], but only recently intakes of the individual carotenoids, which have hypothetical different health effects, have been assessed. Lutein (L) is one of the most widely found carotenoids distributed in frequently consumed fruits and vegetables, and is one of the predominant carotenoids in human plasma [22]. Its presence in human tissues is entirely of dietary origin. Distribution of L among tissues is similar to other carotenoids but, along with zeaxanthin (Z), is found selectively at the centre of the retina, being usually referred to as macular pigment. Current interest in the human macular pigment (MP), consisting of L and Z, is driven largely by its possible association with a reduced risk for age-related macular degeneration (AMD). Increased risk of AMD may result from low levels of L and Z (macular pigment) in the diet, serum or retina, and excessive exposure to blue light [4, 12, 17, 28]. Recent evidence introducing the possibility that L and Z may protect visual function [25, 40, 42] has stimulated the proliferation of supplements containing them [4, 24, 33]. High intakes of fruits and vegetables and of carotenoids are associated with a lower risk for a variety of chronic diseases. Results from observational studies have suggested a possible association between diet, serum L concentration and disease risk [7, 9, 23, 27, 38]. This has enhanced interest in dietary intakes and assessment of status for this carotenoid. It is therefore important to test the validity of a brief dietary questionnaire (FFQ) that assesses these intakes in order to identify a well-developed nutrient specific assessment tool. FFQs are a practical tool for the measurement of food consumption patterns in large surveys, and are widely used both for their ease of use and relative low cost, and for the quantitative data they can collect, but they must be validated against another accurate method of assessment [50]. The 24 h recall may not provide a good performance for investigating one specific nutrient intake, since multiple days are needed to assess carotenoids with any approximation to usual intake; therefore, dietary record is the preferred method used as a reference standard [50].

The primary aim of this study was to develop an assessment tool for L and Z, and validate it against a 7-day dietary record and corresponding plasma biomarker measurement.

Materials and methods

This study is part of a larger project designed to monitor the dietary intake of L and Z in well-nourished women with preterm delivery and to examine associations of dietary intake with plasma and milk concentrations.

Subjects

The participants were adult volunteers (n = 87), females, aged 20–25 years (mean age 22.7 ± 2.1 years), all normal weight (Body Mass Index 18.5–24.9 kg/m^2), with weight stable for at least the previous 3 months, apparently healthy, with no eye diseases, recruited from a group of students of Pavia University, Italy.

Of the 110 women initially recruited, 23 did not meet the inclusion criteria or failed to complete the dietary questionnaires. Individuals with medical conditions that would either interfere with accurate plasma measurement [52] or potentially affect the metabolism of these carotenoids, as well as peculiar lifestyle factors such as smoking, alcohol consumption, L and Z supplements, intense physical activity, restrictive diet or selector eaters as well as vegetarians [38] were excluded. Written informed consent was obtained from all participants prior to their inclusion in the study, which was performed in accordance with the ethical standards laid down in the appropriate version of the 1994 Declaration of Helsinki and approved by Pavia University Faculty of Medicine Ethical Committee.

Study design

In this cross sectional study, during their first visit subjects were submitted to a clinical examination, blood sample collection and were interviewed by a skilled dietician in order to fill in the FFQ, then they were invited and instructed to complete the 7 day dietary records. At the second visit, the same dietician reviewed the 7-day dietary record with the subject to check its completeness and clarity.

Dietary assessment

Development of food frequency questionnaire

A brief FFQ was developed providing a list of fruit and vegetables typically consumed by the Italian adult population according both to the Mediterranean diet [48] and to the Epic FFQ [34, 35]. From this list consisting of 58 items, we selected all the items for which L and Z content was reported in the United States Department of Agriculture–National Cancer Institute Carotenoids Database [19], ending up with a final list of 30 items (dark green leafy vegetables as well as green peas, summer squash, broccoli, lettuce and corn, etc. and fruits like tangerines, peaches, oranges, etc.) reported in Table 1.

Table 1 Food frequency questionnaire aimed at estimating usual L and Z dietary intake

Food items	Number of servings				Portion size (g)
	per month	per week	per day	Never consumed	
Vegetables					
Broccoli					
Brussels sprouts					
Cabbage					
Carrots					
Collards					
Corn					
Green beans					
Green peas (canned)					
Green turnip					
Kale					
Lettuce (romaine)					
Lettuce (iceberg)					
Soup minestrone					
Spinach					
Squash, winter					
Tomatoes					
Tomato juice (canned)					
Tomato products, puree (canned)					
Vegetable juice cocktail					
Zucchini					
Fruits					
Fruit cocktail (canned)					
Grapefruit					
Melon					
Oranges					
Orange juice					
Papaya					
Peach					
Tangerine					
Tangerine juice					
Watermelon					

L and Z concentrations of the selected items are shown in Table 2, outlining the differences in the content of these carotenoids ranging from 13 to 15,798 μg/100 g edible portion.

The FFQ, aimed at covering the previous month's consumption of L and Z, was pre-tested, being reviewed by a panel of eight dietitians in order to check its comprehensibility.

Validation of food frequency questionnaire

The FFQ was validated by comparing the results obtained with a 7-day dietary record as a reference standard.

Data collection

FFQ

We chose to administer the questionnaire by interview since some problems may rise with self-administration: answers may be incomplete as some respondents will only complete the questionnaire for items they usually eat.

All the interviews were performed at the University Department of Human Nutrition by a highly trained staff (three dieticians) who had received 4 h of instruction and was standardized in assessing recalls. Each dietician interviewed 29 subjects.

Summary of questions aimed at obtaining overall information on the number of servings of fruit and vegetables per day, as well as questions for cross checking the consistency of answers, were asked during the interviews.

The FFQ was administered to the subjects before asking them to complete the 7-day dietary records. Completing the FFQ took about 10 min.

In order to quantify eaten portion sizes, each interviewer was equipped with a color food photography atlas [46], previously validated [47], in which, for every food item, three photographs show different portion sizes (small = B, medium = D and large = F). During the interviews each respondent was asked to quantify all food items consumed in relation to one of

Table 2 L and Z concentrations in food items

Food items	L and Z concentrations (μg/100 g edible portion)
Vegetables	
Broccoli (c)	830
Brussels Sprouts (c)	1,290
Cabbage (r)	310
Carrots (r)	358
Collards (c)	8,091
Corn (c)	1,800
Green beans (c)	700
Green peas (canned)	1,350
Green turnip (c)	8,440
Kale (c)	15,798
Lettuce(r) (romaine)	2,635
Lettuce (r) (iceberg)	352
Soup minestrone (c)	150
Spinach (c)	7,043
Spinach (r)	11,938
Squash, winter (c)	66
Tomatoes (r)	130
Tomato juice (canned)	60
Tomato products, puree (canned)	90
Vegetable juice cocktail	90
Zucchini (r)	2,125
Fruits[a]	
Fruit cocktail (canned)	112
Grapefruit	13
Melon	40
Oranges	187
Orange juice	105
Papaya	75
Peach	57
Tangerine	243
Tangerine juice	166
Watermelon	17

c cooked, *r* raw
[a]All the fruit items have been considered raw

the three photographs or in terms of virtual portions placed in between those shown in the photographs (coded as A, C, E and G).

It is well known that FFQ and food records may have common sources of errors such as reliance upon memory of portion sizes conceptualization and over/under reporting of diet [8]. Therefore, we chose to use the same food photography atlas for both instruments in order to reduce these errors, although this might bias the results of the Bland Altman Plot and reinforce Pearson's correlation.

Frequency of usual food consumption was investigated by inviting the respondents to report their consumption as "never consumed", or in the units of their choice such as "number of occasions" per day, week or month, rather than being restricted to specific frequency ranges with a reference period of "over the past month" as already reported by other authors [31, 38]. Usual L and Z dietary intake was assessed with this quantitative 30-item FFQ during the clinical examination.

Subjects reported that the FFQ was very comprehensive, clear and easy to answer, as well as brief and not stressful.

Dietary record

Each subject was asked to keep a 7-day dietary record, as a reference standard, after the FFQ administration. Participants were carefully instructed on how to complete the dietary diary; recording everything they ate and drank while eating. In order to quantify the portion sizes of the foods and beverages consumed, the subjects enrolled were provided with a personal password to access online software containing the above-mentioned food atlas photographs. They were asked to report the quantity consumed, at the end of each day.

Anthropometric measurements

Women were submitted to a clinical examination, during which anthropometric measurements were taken. Standing height (without shoes) was measured to the nearest 0.1 cm with a stadiometer; body weight was measured in underwear with a calibrated mechanical balance accurate to ±0.1 kg.

Plasma measurements

At the first visit, after an overnight fast, blood samples were collected for determination of plasma L and Z concentrations. Blood samples were collected in tubes containing 0.1% EDTA, were stored in a cooler with ice packs at 4°C and were transported to the laboratories within 4 h. Blood was centrifuged for 20 min at 4°C and 1,430*g* to separate plasma, stored at −80°C and afterwards transported on dry-ice to the laboratories for analysis. Analysis of plasma L and Z concentrations were measured with a reversed-phase HPLC method as described by Yeum et al. [51]. The peaks were not separated and they are referred to as L and Z concentrations. Within-assay L and Z concentration measurements were found to be highly repeatable. The percentage recovery of the internal standard varied between 86 and 99% and the detection limit was ≤0.02 μmol/l. Intra-assay precision was 3%.

Data analysis

The FFQ and the 7-day dietary record were coded by the dieticians and the results analyzed using the United States Department of Agriculture–National Cancer institute Carotenoids Database [19], a considerably updated carotenoid food content database.

L and Z dietary intake are reported together because their content is combined in the nutrient database used.

Since an Italian database on carotenoid content is not available, we decided to use the above mentioned database, even though carotenoid content vary slightly from country to country.

The statistical software package SPSS (version 13) was used for data analysis. Means and standard deviations were calculated for L and Z intakes measured by the FFQ and the 7-day dietary record. The correlation between the two dietary assessment methods was computed with Pearson's correlation coefficient. In addition, the agreement between the two methods was assessed as proposed by Bland Altman [2]. Pearson's correlation coefficient was also used to assess associations of dietary intake of L and Z with their plasma concentrations.

Results

Sample

Table 3 presents a description of the study population: 87 females, non-smokers, and university students, aged 20–25 years. According to BMI they were all normal weight. Dietary intake and plasma concentrations of L and Z are also summarized in Table 3.

Dietary intakes

Mean dietary L and Z intakes were 1,107 ± 113 μg/day and 1,083 ± 116 μg/day computed from the FFQ and the 7 day dietary record, respectively. A highly significant correlation coefficient ($r = 0.94$, $P < 0.0001$, 95% CI 0.91–0.96) was obtained between the intakes assessed by the FFQ and the 7-day dietary record. In order to see, at what extent the FFQ and the 7-day dietary record were in agreement, data were plotted in a scatter diagram (Fig. 1) and the differences between the measurements by the two methods were analyzed. We found that all our data points were quite near to the line of equality (or correspondence). The mean difference in intake by the two methods (−24.5 ± 38.3 μg/day) was negligible, so we could say that on average the two methods agree considerably. Then, to consider how well the two methods were likely to agree for an individual, the differences between L and Z intake assessed by the 7 day dietary records, L and Z intake assessed by FFQ were plotted against the average of the two measurements (Bland Altman plot) (Fig. 2) and the 95% limits of agreement were calculated. We obtained a range, which was −50.6 to 99.6 μg/day, which is acceptable for the assessment of individual L and Z intake.

Table 3 Descriptive characteristics of the sample ($n = 87$)

Variable	Value (mean ± SD)
Age (years)	23.3 ± 2.8
BMI (kg/m^2)	22.7 ± 3.1
Plasma L and Z (μmol/L)	0.33 ± 0.09
FFQ dietary intake of L and Z (μg/day)	1,107 ± 113
7-Day DR[a] dietary intake of L and Z (μg/day)	1,083 ± 116
Fruit (servings/day)	1.17 ± 1.01
Vegetables (servings/day)	1.20 ± 1.03

[a]7 Day dietary record

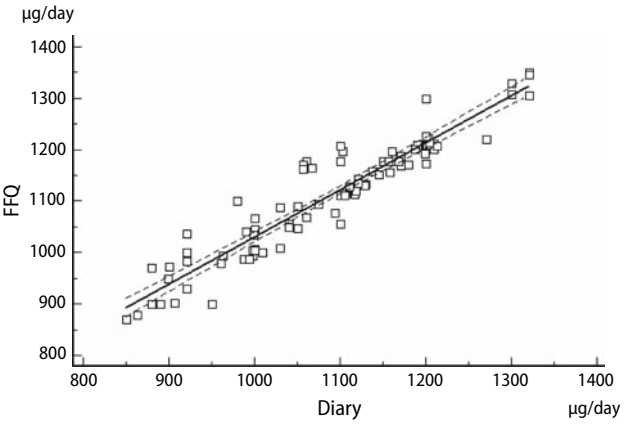

Fig. 1 Correlation between L and Z intakes assessed by the FFQ and the 7-day dietary record (Diary)

Plasma data

Mean plasma L and Z concentration was 0.33 ± 0.09 μmol/l. Plasma L and Z concentrations

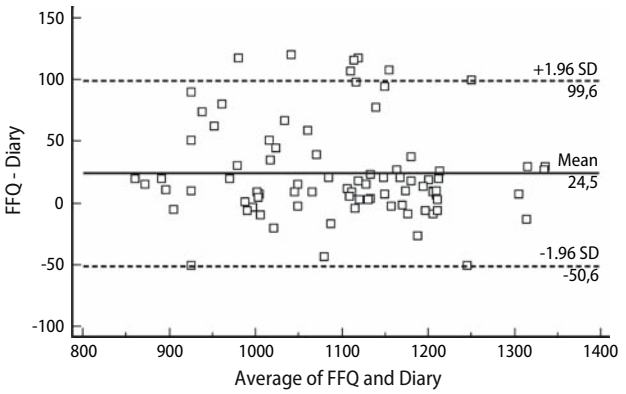

Fig. 2 Differences between L and Z intakes assessed by the FFQs and the 7-day dietary records (FFQ-Diary) plotted against the average of the two measurements (Bland-Altman Plot)

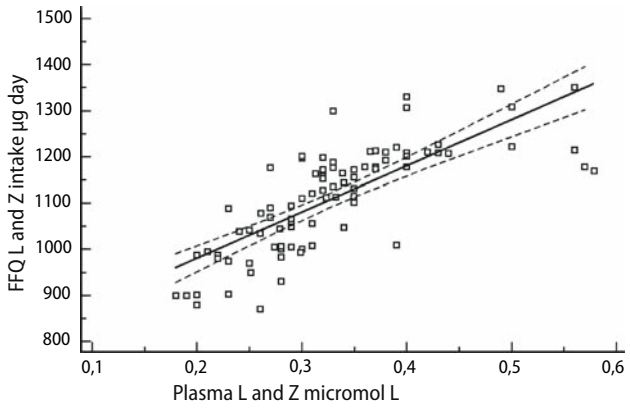

Fig. 3 Correlation between plasma values and dietary intake of L and Z assessed by the FFQ

were significantly correlated with the dietary intakes obtained from FFQ ($r = 0.76$, $P < 0.0001$; 95% CI 0.66–0.84) (Fig. 3). Such correlation was reinforced by the quite high level of agreement found between the two dietary assessment methods. Plasma L and Z concentrations correlated significantly with servings per day of fruit ($r = 0.54$, $P < 0.01$) and vegetables ($r = 0.61$, $P < 0.001$), while no correlations emerged with age, BMI and body weight.

Discussion

This study was conducted in order to validate a brief FFQ assessing L and Z intake in a sample of 87 Italian women aged 20–25 years. The population in the present study may be small but is homogenous in terms of age, BMI, educational level, race/ethnicity, lifestyle habits, etc. thereby minimizing the effect of non-dietary determinants that in larger epidemiological studies may influence serum L and Z concentration [38]. Nevertheless, we recognize that our sample may not fully represent the future study population, but there are many difficulties inherent to the design of a validation study involving pregnant/postpartum women.

On the other hand, we are still confident regarding the validity of the FFQ, since our sample was selected as similar to the general population in terms of age, sex and lifestyle habits; it is therefore useful for future research aimed at studying the protective role of these compounds in well nourished pregnant women and in their newborns.

The 7-day dietary record method was used as the primary reference method to validate the FFQ, although its limitations for individual assessment of habitual intakes are well known. However, no dietary assessment method can be regarded as a "gold standard" and it may be unrealistic to accord special status to any method [50].

The dietary record method should be the first method of choice for validating FFQs [50]. Although 24-h recalls are less demanding for the participant than dietary recording and less likely to influence the actual diet of the subjects, their sources of errors tend to be more correlated with the error in dietary questionnaires (e.g., reliance upon memory conceptualization of portion sizes and distortion of reported diet) [8]. When used as a reference method, dietary records should be kept for a sufficient number of days to represent average intake. Using data from two studies, Stram et al. [44] presented calculations to determine the "ideal" number of days of dietary recording to use in a validation study. They concluded that, in most settings, the optimal study design will rarely require more than four or five diet records per subject. There is some evidence [8] that increasing the number of days of recording in the reference method improves the apparent validity of a questionnaire. It would appear that efforts to increase the duration of recording in the reference method provide a better measure of habitual intake, which is generally more similar to the type of information generated by FFQ. Therefore, we chose to record dietary consumption for seven days. The mean intake of L and Z estimated by the FFQ is very similar to the mean value obtained with the 7 day dietary record and Pearson's correlation coefficient is very high, confirming the validity of this brief FFQ, as previous studies have already reported in assessing other micronutrient intakes [1, 15, 41]. In addition, the Bland Altman plot shows that the two methods are very likely to agree for individual intake, since the range obtained for the 95% limits of agreement was −50.6 to 99.6 µg/day, which may well be very satisfactory for the assessment of individual L and Z intake. Furthermore, the dots are equally distributed in the plot and there is no distortion in one or the other direction. Better results were achieved by using the set of photographs instead of standard portions in both dietary assessment methods [5], thus enhancing the performance of the methods. The results of this study demonstrated a clear association between dietary L and Z intake and plasma concentration ($r = 0.76$, $P < 0.0001$; 95% CI 0.66–0.84). Our dietary assessment tool (brief FFQ) gives a reliable estimate of usual dietary intake; this may be due in part to an improvement in the quality and quantity of food content data for L and Z, as already observed by other authors [38]. Usual dietary intake assessed over the previous month is especially important when estimating carotenoid intake, due to the high day to day variability in intake of these compounds, although there may be many sources of error in the use of an FFQ, such as the restrictions imposed by fixed

lists of food, portion size estimations, the cognitive challenge of reporting foods consumed over a broad time span such as the past month [50] and the limited ability to differentiate between cooked and raw vegetables, which affects carotenoid bioavailability [49].

In addition our results show that the average daily intake for L and Z is similar to that obtained by Curran-Celentano et al. [12] in a group of 280 healthy adult volunteers aged between 18 and 50 years in the Indianapolis area and bordering counties (1,101 ± 838 μg/day), as well as that measured by Hammond et al. [18] in a group of 280 obese subjects aged 36 ± 7.7 years (1,198 ± 904 μg/day). It was higher (1,347 ± 891 μg/day) in a heterogeneous community based sample of adults aged 18–92 years recruited and examined at three US sites (n = 2,786) by Rock et al. [38]. L and Z dietary intake data from the Third National Health and Nutrition Examination Survey, show an average intake of 2,000–2,300 μg daily for men and 1,700–2,000 μg daily for women in the USA [14]. Correlation coefficients between plasma L and Z concentrations and servings per day of fruit (r = 0.54, $P < 0.01$) and vegetables (r = 0.61, $P < 0.001$) appear to be sufficient as an indicator of L and Z dietary intake, suggesting that a regular intake of selected fruit and vegetables leads to a progressive increase in plasma L and Z concentrations. Moreover, plasma concentration of these carotenoids in our sample is comparable to that measured by Johnson et al. [21] (0.37 ± 0.05 μmol/l) in seven healthy adults of both sexes aged 33–54 years as well as that measured by Hammond et al. [18] (0.38 ± 0.17 μmol/l) and by Curran-Celentano et al. [12] (0.28 ± 013 μmol/l), respectively in a sample of obese subjects and in a large group of adult volunteers. On the other hand, lower values were found by Molldrem et al. [30] (0.23 ± 0.07 μmol/l) in nine healthy male and female adults aged 23–28 years participating in a randomized blinded 3 × 3 cross over intervention study, as well as the one reported by Rock et al. [38] (0.22 ± 0.12 μmol/l) and by Broekmans et al. [6] (0.17 ± 0.07 μmol/l and 0.19 ± 0.09 μmol/l in males and females, respectively) in a group of 376 volunteers aged 18–75 years.

Finally the fact that no correlations between plasma L and Z concentrations and age, BMI, height and body weight emerged in this study can be explained by the fact that our sample is homogeneous.

L and Z are important nutrients not only for ocular health [7, 9, 25, 27, 40, 42] but also for the prevention of cardiovascular disease, stroke, and lung cancer [10, 17, 23, 28, 36] as well as for skin protection in conditions attributed to excessive ultraviolet (UV) light exposure [13, 26, 32, 43].

In conclusion, FFQ is useful to assess habitual L and Z intake in Italian adult women and therefore to examine the use of these carotenoids as a biomarker of exposure in clinical practice, providing some rationale for assessing their relationship with human health as well as their potential implication within the context of evidence-based medicine [17].

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