International Journal of Food Sciences and Nutrition.

DOI: 10.3109/09637486.2016.1147020 (versión aceptada el 24 enero 2016)

Assessment of dietary lutein, zeaxanthin and lycopene intakes and sources in the Spanish Survey of Dietary Intake (2009-2010)

Rocío Estévez-Santiago¹, Beatriz Beltrán-de-Miguel², Begoña Olmedilla-Alonso¹

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

² Facultad de Farmacia. Universidad Complutense de Madrid (Spain)

Corresponding author:

B. Olmedilla-Alonso. ICTAN-CSIC. C/ José Antonio Novais, 10. 28040-Madrid (Spain).

Phone: +34- 91549 2300. Fax: (+34) 91 549 3627. E-mail address:

Bolmedilla@ictan.csic.es

Abstract

We assessed the intake and major dietary sources of lutein, zeaxanthin and lycopene (non-provitamin A carotenoids) in Spain using food consumption data from the Spanish National Dietary Intake Survey (2009–2010). 3-day diaries and one 24-h recall were used to collect dietary data and a software application that includes HPLC data was used. Average intake of those carotenoids was 4290.8 µg/day (67.1% total carotenoid intake), mainly from vegetables (3414.0 µg/day), followed by fruits (393.5 µg/day), oils/fats (204.0 µg/d), and eggs/egg products (170.0 µg/day). Main sources of lutein and zeaxanthin were vegetables (62.9% total diet, 1235.2 µg/person/d). Lycopene intake was 3055.6 µg/day (71.2% of non-provitamin A carotenoids), mainly from tomato and by-products (86.3%) and watermelon. Red- and orange-colored fruits and vegetables were the major contributors of non-provitamin carotenoids (3219.0 µg/person/d). Balanced diets should favor fruits and vegetables over other dietary sources (oils, eggs, processed foods) that contain components to be consumed with moderation.

Key words: food consumption survey, non-provitamin A carotenoids, bioactive compounds.

Introduction

Lutein, zeaxanthin and lycopene (non-provitamin A carotenoids) are plant pigments that belong to the well-known group of carotenoids. They are provided by the diet, specially by fruits and vegetables. Together with the provitamin A carotenoids and other bioactive compounds, they are partly responsible for the protective role in human health that are associated with the consumption of fruits and vegetables (Seddon *et al.*, 1994, Shashirekha *et al.*, 2015, Liu 2013, Jomova and Valko 2013). Dietary lutein and zeaxanthin intake and the presence of these compounds in blood and/or tissues have been associated with a lower risk for age-related diseases, such as macular degeneration or cognitive deterioration (Rao *et al.*, 2006, Min and Min 2014, Granado *et al.*, 2003, Moeller *et al.*, 2008). Lutein and zeaxanthin are concentrated in retina, where they constitute what it is known as the macular pigment [MP] which protects the photoreceptor cell layer from light damage by filtering blue light, and where they act as scavengers of radical oxygen species (Zampatti *et al.*, 2014, Kijlstra *et al.*, 2012, Snodderly 1995). Lutein and zeaxanthin concentrations in the retina increase upon the consumption of lutein-rich foods (especially spinach, kale and egg yolk), lutein fortified foods (i.e. egg products) and lutein supplements (Granado *et al.*, 2003, Shardell *et al.*, 2011, Thurnham 2007). Lycopene, another dietary carotenoid present in human blood and tissues, is found in a limited number of foods, specially in tomato and tomato products, and is present in many processed foods in which tomato is used as an ingredient; it is also present in certain fruits (watermelon, pink grapefruit, papaya, persimmon, etc.) (Naz *et al.*, 2014). Lycopene is the most efficient singlet oxygen quencher *in vitro* (Stahl and Sies 2003) and this biological activity seems to be of interest for human health (i.e. cardiovascular health) (Perveen *et al.*, 2015, Rao *et al.*, 2006). Evidence from epidemiological studies suggests that high consumption of tomato products or lycopene is associated with a significantly lower risk of numerous cancers (Giovannucci 1999, Gann *et al.*, 1999).

The biological activity of these carotenoids in the human body (Stahl and Sies 2003) and the increase in the number of human studies involving these compounds in recent decades (Krinsky and Johnson 2005, Fiedor and Burda 2014) highlight the importance of the knowledge of the contents of individual carotenoids in foods for a better understanding of their role in the relationship between diet and health. However, although there has been an increase in the number of food composition tables that provide data on the contents of these three carotenoids in foods (West and Poortvliet 1993, Holden *et al.*, 1999, Holden *et al.*, 1998, O'Neill *et al.*, 2001), to date, the specific intake of these compounds in relation to health status is evaluated at the population level in the USA.

In Spain, the Spanish Agency for Food Safety and Nutrition (AESAN) published the *Encuesta Nacional de Ingesta Dietética* [Spanish Dietary Intake Survey] (ENIDE) for 2009-2010 (AESAN, 2011). For the assessment of micronutrient intake, the ENIDE relied on the *Base de Datos Española de Composición de Alimentos (BEDCA)* (Spanish Food Composition Database), which is included in the food databases of the EuroFIR association (European Food Information Resource). The BEDCA does not include lutein, zeaxanthin or lycopene concentrations in the foods and, thus, the ENIDE report did not provide individual data on the dietary intake of those carotenoids.

In a previous paper (Beltran-de-Miguel *et al.*, 2015) we assessed the intake of provitamin A carotenoids using food consumption data from the ENIDE survey (AESAN 2011) and a database of carotenoid content in foods designed by our group (Beltrán *et al.*, 2012). The objective of the present study was to expand the information provided in the ENIDE by assessing the intake of lutein, zeaxanthin and lycopene; the non-provitamin-A carotenoids most frequently studied in terms of the relationship between diet and health, and identifying their major dietary sources in the Spanish population. The dietary intake of these

compounds was calculated for the diet as a whole and according to food groups, with the fruit and vegetable group broken down according to color.

Subjects and methods

The dietary intake of non-provitamin carotenoids in the Spanish population was evaluated using the food consumption data provided by the ENIDE survey, part of a study conducted in 2009 and 2010 by the AESAN, on behalf of the Spanish Ministry of Health, Social Services and Equality, to determine dietary intake patterns in the adult Spanish population. The ENIDE is a cross-sectional survey involving a sample of non-institutionalized individuals (n= 3000), aged 18-64 years, that was designed to be representative of the Spanish population (AESAN, 2012). Possible participants were randomly selected using a stratified approach that took into account demographic characteristics, and were invited to participate by phone call followed by a letter with the study information. All participants gave their written informed consent. The ENIDE study took into account demographic characteristics (gender, age, geographical zone, family size and level of education), lifestyle, diet and seasonal consumption, collecting data from the same number of participants in each of the four seasons of the year. The food consumption data were obtained from a 24-h recall completed by each participant with the aid of a trained interviewer, as well as a 3-day diary, which together constituted a record of more than 12000 days of consumption (AESAN, 2012). Using this information, the ENIDE survey revealed the distribution of daily consumption of foods (n>400), classified into 12 groups according to intake. Each food (raw) is expressed in grams or mL (in the case of liquids) per person and per day, considering the overall population (consumers and nonconsumers) (AESAN, 2012).

In this study, data on the mean consumption of foods (g/day) based on the ENIDE survey (AESAN, 2011) were used to calculate the intake of the non-provitamin A

125 carotenoids: lutein, zeaxanthin and lycopene. The ENIDE survey gathered information on the
126 consumption of mixed meals (recipes), recording both the amounts consumed by the
127 participants and the way in which the meals were prepared. This information was transformed
128 into data on the foods and amounts in each recipe, and we used these data to assess the dietary
129 intake of lutein, zeaxanthin and lycopene. The data on the foods and amounts consumed were
130 introduced into a specific software application for carotenoids (Estévez-Santiago *et al.*, 2013)
131 that includes a carotenoid database (Beltrán *et al.*, 2012). This carotenoid database contains
132 information on the major dietary carotenoids present in foods, with data generated entirely by
133 high-performance liquid chromatography (HPLC) (Beltrán *et al.*, 2012, Granado *et al.*, 1992,
134 Olmedilla *et al.*, 1993) using an analytical procedure that is considered to be highly
135 acceptable (West and Poortvliet 1993). The foods included, mainly from the plant kingdom,
136 are major contributors to the intake of carotenoids in Europe (Granado *et al.*, 2007, O'Neill *et*
137 *al.*, 2001). The food groups included in the software are: fruit, vegetables, oils and fats,
138 snacks, nonalcoholic beverages, milk and dairy products, eggs and egg products, sauces and
139 herbs and spices. The individual carotenoid content of each of these items was multiplied by
140 the amount consumed of edible portion (g/person/day) and this provided an estimate of the
141 carotenoid contributed by the food. The sum of the carotenoid intakes from these foods
142 enabled the calculation of individual and total carotenoid intakes, as well as their relative
143 contribution. Data on the contribution of the different food groups to energy intake among the
144 Spanish population (in the ENIDE) were previously assessed and published (Beltran-de-
145 Miguel *et al.*, 2015) and were used to assess said intake expressed in terms of dietary energy
146 density (lutein, zeaxanthin and lycopene per 1000 kcal). The data on carotenoid
147 concentrations were those corresponding to raw and cooked foods, depending on how they are
148 usually consumed.

The major food sources of lutein, zeaxanthin and lycopene in the diet of the Spanish population were determined, and the contribution of the group comprised of fruits, vegetables and other foods of plant origin to the intake of these bioactive compounds is presented for individual items and grouped according to their color (white/yellow, green, red/orange).

Results

The contribution of lutein, zeaxanthin and lycopene in the total diet of the adult Spanish population, and the contribution of the different food groups to said intake are shown in table 1. The sum of these non-provitamin A carotenoids is also included, as are previously published data on the intake of provitamin A carotenoids (β -carotene, α -carotene and β -cryptoxanthin) (Beltran-de-Miguel *et al.*, 2015) to assess the total intake of the major dietary carotenoids. In this table, the lutein and zeaxanthin columns provide intake data of these two carotenoids when they were determined separately, and the lutein-zeaxanthin column shows their combined values obtained from the literature (when a joint value was reported) (see database in Beltrán *et al.*, 2012). The column total L+Z reports the total lutein and zeaxanthin intake. The mean total dietary intake of non-provitamin A carotenoids was 4290.8 $\mu\text{g/d}$ (67.1% of the total carotenoids) and was mainly supplied by vegetables (3414.0 $\mu\text{g/d}$) and fruits (393.5 $\mu\text{g/d}$), followed by the group of oils and fats (204.0 $\mu\text{g/d}$). Eggs and egg products constitute the only source of these carotenoids of animal origin (170.0 $\mu\text{g/d}$).

Dietary intakes of non-provitamin A carotenoids are shown in table 2. Dietary intake of lutein (704.0 $\mu\text{g/person/d}$) was higher than that of zeaxanthin (55.0 $\mu\text{g/d}$). The total intake of lutein and zeaxanthin (1235.2 $\mu\text{g/d}$) was approximately 2.5-fold lower than that of lycopene (3055.6 $\mu\text{g/d}$). Lycopene represented 71.2% of the non-provitamin A carotenoids analyzed in the Spanish diet and it was provided mainly by one vegetable – tomato (86.3% of the total lycopene intake) and a fruit, watermelon (10.6% of the total dietary lycopene). The

main source of lutein and zeaxanthin are vegetables (62.9% of the total dietary intake) and the contribution of the groups of oils and fats and, to a lesser extent, that of eggs and egg products (16.5% and 5.2%, respectively).

The contribution of the different vegetables and fruits (g/d) to the intake of lutein, zeaxanthin and lycopene is shown in table 2, as are the amounts of total and individual non-provitamin A carotenoids supplied daily by each of these foods. Spinach (182.7 µg/d, total L+Z) and curly kale (173.8µg/d, total L+Z) were the major sources of lutein and zeaxanthin which, together, provided 28.9% of the total intake of these carotenoids in the diet as a whole. Together peppers and Swiss chard, they constituted 35.4% of the total L+Z intake. Fruits provided 5.2% of the dietary lutein and zeaxanthin intake, with orange as the leading contributor by a small margin (33.4 µg/d of L+Z), as it was consumed in greater amounts (34.6 g/d), supplying 52.4% of the L+Z contributed by the group of fruits.

Finally, table 3 shows the lutein, zeaxanthin and lycopene intake provided by fruits and vegetables grouped according to their color as white/yellow, red/orange and green. When compared to foods of other colors, the red and orange fruits and vegetables were, by far, those that contributed the greatest amount of non-provitamin A carotenoids when considered as a whole (75.0 %), and all the dietary lycopene. However, considering non-provitamin A carotenoids individually, lutein and zeaxanthin were mainly supplied by green fruits and vegetables (51.1%), whereas red/orange colored items provided 13.2% and those of white/yellowish color (21.9%). The fruits and vegetables of red/orange color supplied 97.1% of the lycopene intake.

Discussion

In this report, we present the first data on lutein, zeaxanthin and lycopene intakes in a Spanish population (a representative sample at the national level), which were calculated on

the basis of a direct dietary survey using a methodology for the analysis of the intakes of individual carotenoids. This survey methodology is of the type chosen by EuroFIR (Martinez Burgos *et al.*, 2009) in the attempt to harmonize and standardize food consumption data from different countries.

Until now, information on the intakes of these carotenoids in Spain has been very limited, and the available data, national and international, have been provided by studies with considerable differences in methodology that affect the comparability of the results obtained. This is especially true for databases of carotenoid composition in foods and the types of dietary questionnaires employed, a circumstance that could explain the very different intake levels found in the literature (Kant *et al.*, 2015, USDA-ARS, 2012, O'Neill *et al.*, 2001). Previous data on the intake of non-provitamin A carotenoids in the Spanish population, from fruit and vegetable consumption (major contributors to their intake) but not from the total diet were based on calculations of consumption frequencies obtained from the household budget surveys carried out by the Spanish Institute of Statistics in 1964, 1980 and 1996 and from a food consumption survey carried out by the Ministry of Agriculture in 2004 (Granado *et al.*, 2007) (table 4). The food composition data reported in their studies (Granado *et al.*, 1992, Olmedilla *et al.*, 1993) were included and expanded in the carotenoid database employed in the present study (Beltrán *et al.*, 2012).

To compare our data with those from Granado *et al.* (Granado *et al.*, 2007), we consider only the fruit and vegetable intakes (476 g fruit+vegetables/p/d; 263 g vegetables/p/day and 213 g fruit/p/d) according to the Spanish food consumption reported by the ENIDE (AESAN, 2011) as contributors to lutein, zeaxanthin and lycopene intake. The lutein and zeaxanthin intake (840.2 µg/p/d) and lycopene intake (2967.0 µg/p/d) are higher in the present study, in both cases, almost double the values obtained with the 2004 food consumption survey (453.1 µg lutein and zeaxanthin /p/d and 1162.0 µg lycopene/p/d)

(Granado *et al.*, 2007). This difference may be due to the increase in the varieties of fruits and vegetables recorded in the ENIDE (around 30 vegetables and 20 fruits) compared to the 12 vegetables and 16 fruits considered in the calculation of the survey of 2004, as well as to the availability of more data on carotenoid composition in the present study. For example, for the lycopene calculations, the 2004 survey did not take into account the contribution of sauces and juices (i.e., tomato sauce and tomato juice). In the survey of 2004, those items represented at least 89% of the total consumption of fresh fruit and vegetables in Spain (Granado *et al.*, 2007).

The intake of non-provitamin A carotenoids from fruits and vegetables in proportion to the intake of total carotenoids (67.3%) in the Spanish population is higher than that reported with the data from the survey of 2004, in which it represented 50% of the total intake (Granado *et al.*, 2007). Fruits supply similar amounts of carotenoids with and without provitamin A activity, but vegetables are richer in non-provitamin A carotenoids (68.8 %) (Table 1). Lutein and zeaxanthin represent the 14.8% of the total carotenoids from fruits and vegetables, which is similar to the percentage obtained with the 2004 survey (14%).

Intakes from the U.S National Health and Nutrition Examination Survey (NHANES 2009-2010) were 5260.0 µg/day for lycopene and 1360.0 µg /day for lutein + zeaxanthin (which, expressed in mg per 1000 kcal, are 2530.0 µg lycopene/1000 kcal and 650.0 µg lutein plus zeaxanthin/1000 kcal). The lycopene intake in the U.S. is much higher than in Spain (3055.6 µg/d) and the lutein+zeaxanthin intake (1235.2 µg/d) is slightly higher. These differences could be due to an underestimation of the lycopene and of the lutein+zeaxanthin intakes as the foods grouped as “others” were not taken into account in the estimation of these intakes. That group included prepared foods, which can include tomato or lycopene as a color additive and constitute relevant sources in some countries (e.g., Belgium) (Vandevijvere *et al.*, 2014), as well as foods derived from grains, which can contain lutein+zeaxanthin (e.g.,

maize, although the consumption of these foods is of little importance in the Spanish diet). Although there are few data in Spain on the food composition of the many processed or ready-to-eat foods that are commercially available and the consumption of which is on the rise (Hoffman and Gerber 2015) their contribution should not be overlooked because it could result in the underestimation of the dietary carotenoid intake.

The lutein and zeaxanthin intake in this study in a Spanish population aged 18 - 64 years (1230 µg/p/d) is similar to that found in a recent study in apparently healthy individuals (n=108; aged 20-35 and 45-65 years), in which the dietary intake was 1170 µg/p/d (Olmedilla-Alonso *et al.*, 2014), and was measured using 3-day food records. 24-h recalls and the same food database employed in the present study. However, the total dietary lutein and zeaxanthin intake is much lower in this study than that found in European subjects (n=400) more than a decade ago, where the intake in the Spanish subgroup (n=80) was 3250.0 µg /day (range: 1800.0 – 4300.0 µg/d) (Olmedilla *et al.*, 2001), which contrasts with the 1230.0 µg /d of this study. The opposite occurs with lycopene, the intake of which (3060.0 µg /p/d) is higher than in the aforementioned Spanish subgroup (median 1640.0 and range 500.0 to 2640.0 µg /p/d). In the two studies, the food composition data was identical for most of the foods; however, the European study was based on a semiquantitative food frequency questionnaire (FFQ), whereas in the present study, 3-day food records were employed. FFQ's have been reported to overestimate carotenoid intake (George *et al.*, 2012), especially that of lutein and zeaxanthin, when the findings with these two tools are compared (Granado-Lorencio *et al.*, 2006).

In the present study, sources of lutein and zeaxanthin like the eggs and oils are included, but were not evaluated in the study of Granado *et al.* (Granado *et al.*, 2007). Specifically, olive oil, widely used as a cooking oil in Spain (AESAN, 2011), provides 16.5% of the total lutein and zeaxanthin intake. However, it is important to stress the benefits of

fruits and vegetables in comparison to other sources of carotenoids, such as oils, because they have a high nutrient density, accompanied by a low energy density (defined as kcal per gram of food). Oil has an energy density 20 times higher than fruits and vegetables and, thus, the health recommendation for oil consumption (30-40 g/day) would detract from the importance of this food if compared with fruits and vegetables in any health campaign. In this respect, the lutein and zeaxanthin density ($\mu\text{g}/1000 \text{ kcal}$) in vegetables is maximal (8.95 mg/1000 kcal), a fact that makes promotions of the consumption of this food group, which provides other bioactive components of interest, more convincing. Fruits and vegetables should be given priority in the design of balanced diets and preference over other carotenoid-rich foods (sauces and processed foods) (Vandevijvere *et al.*, 2014), the consumption of which should be limited because of the possible harmful effects of excessive amounts of some of their ingredients (salt, fat, cholesterol, etc.).

Among the fruits and vegetables, spinach and curly kale provide 46% of lutein and zeaxanthin intake, which corresponds to 28.9% of the total dietary intake. However, we must not overlook the contributions of other vegetables, although the consumption of some of them ranges from $<1 \text{ g/day}$ (e.g. watercress, lamb's lettuce), because together they provide 7.1% of the lutein+zeaxanthin in the total diet. With respect to lycopene, one vegetable (tomato) and one fruit (watermelon) are its main sources, of which has also been reported in other studies (Granado *et al.*, 1996, Rodriguez Amaya 2001).

The mean dietary intake of fruits and vegetables in this study (392 g/day: 213 g/day fruit and 179 g/day vegetables [edible portion, excluding potatoes and tubers]) practically meets the amounts of fruits and vegetables recommended by the WHO (400 g/day, excluding potatoes and starchy tubers) (WHO/FAO, 2003). However, it does not meet the amounts recommended by the World Cancer Research Fund International (WCRF, 2007) for public health goals: 600 g/p/day of a range of non-starchy vegetables and fruits that comprises items

of different colors, including red, green, yellow, white, purple and orange. On the other hand, the different distributions of these carotenoids in fruits and vegetables grouped according to their color supports the need to explain to the general population the benefits derived from diets that include not only a high amount of fruits and vegetables, but that these foods should be of a variety of colors (Heber and Bowerman 2001); this recommendation is being issued by several organizations (WCRF 2007, USDA 2011).

There are no dietary reference intakes for the bioactive components, as their absence from the diet is presumably not deleterious to health (e.g., does not cause a deficiency syndrome) (Harper 1999), but, mainly on the basis of epidemiological and intervention studies and the biological activities of these compounds, suggestions for intake levels that could decrease the risk of several chronic diseases have been made. For lutein, an intake of 6 mg/day has been suggested to decrease risk of some chronic diseases (Granado *et al.*, 2003). This level is much higher than the lutein intake of the Spanish population, which represents only 14% of this suggested intake. On the other hand, according to the observed safe level risk assessment method, the evidence of safety is strong at intakes up to 20 mg/d for lutein and 75 mg/d for lycopene (Shao and Hathcock 2006), amounts difficult to achieve by dietary means without the incorporation of fortified foods.

Conclusions

The identification of the major sources of lutein, zeaxanthin and lycopene and their intakes in the Spanish population will be useful for decisions in the public health setting, as those carotenoids account for more than half of the total dietary carotenoid intake in said population. Moreover, it is of the utmost importance that the efforts around the world to harmonize the results continue, with a standardization of the methodology and databases

utilized in dietary studies. This would enable a more reliable approach to the establishment of adequate reference intake levels related to the physiological effect of these compounds.

To date, the recommendations of international organizations (WHO, WCRF) with respect to increasing the consumption of fruits and vegetables (with a variety of colors) is essential for a higher intake of lutein, zeaxanthin and lycopene in the Spanish population, as this food group provides nearly 90% of these carotenoids. The mean dietary intake of fruits and vegetables in the Spanish population meets the 400 g fruit and vegetable daily intake recommended by the WHO (WHO/FAO, 2003). Specifically, lycopene is ingested mainly from red/orange fruits and vegetables, almost exclusively tomato and watermelon; and lutein and zeaxanthin are found in green-colored foods, mainly spinach and curly kale.

Declaration of interest

This study was supported by grant from Acción Estratégica en Salud of the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación Tecnológica, Spain) (grant no. PS09/00681). Rocío Estévez-Santiago is the recipient of a JAEPredoc grant from the Spanish Consejo Superior de Investigaciones Científicas (CSIC), awarded under the program of the Junta para la Ampliación de Estudios, cofinanced by the European Social Fund (ESF).

References

AESAN, Agencia Española de Seguridad Alimentaria y Nutrición. 2011. Datos de consumo de alimentos (gramos/día) a partir del estudio ENIDE. 2011. Available at: http://www.aesan.mspsi.gob.es/AESAN/docs/docs/evaluacion_riesgos/datos_consumo/ENIDE.pdf. Accessed on July 2014.

346 AESAN, Agencia Española de Seguridad Alimentaria y Nutrición. 2012. Evaluación
 347 Nutricional de la Dieta Española II. Micronutrientes. Available at:
 348 http://aesan.msssi.gob.es/AESAN/docs/docs/evaluacion_riesgos/estudios_evaluacion_nutricional/Valoracion_nutricional_ENIDE_micronutrientes.pdf. Accessed on July
 349 2015. Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). 2012.
 350 Evaluación Nutricional de la Dieta Española II. Macronutrientes. Available at:
 351 http://www.aesan.mspsi.gob.es/en/AESAN/web/evaluacion_riesgos/subseccion/enide.shtml
 352 and at: http://aesan.msssi.gob.es/en/AESAN/web/notas_prensa/presentacion_enide.shtml. Accessed on July 2014.
 353
 354
 355 BEDCA, Base Española de Datos de Composición de Alimentos. Agencia Española de
 356 Seguridad Alimentaria y Nutrición AESAN. 2006. Available at: <http://www.bedca.net/>
 357 Accessed July 2015.
 358 Beltran-de-Miguel, B., Estevez-Santiago, R. & Olmedilla-Alonso, B. 2015. Assessment of
 359 dietary vitamin A intake (retinol, alpha-carotene, beta-carotene, beta-cryptoxanthin)
 360 and its sources in the National Survey of Dietary Intake in Spain (2009-2010). *Int J*
 361 *Food Sci Nutr* , 66, 706-712.
 362 Beltrán, B., Estévez, R., Cuadrado, C., Jiménez, S. & Olmedilla Alonso, B. 2012. Base de
 363 datos de carotenoides para valoración de la ingesta dietética de carotenos, xantofilas y
 364 de vitamina A; Utilización en un estudio comparativo del estado nutricional en
 365 vitamina A de adultos jóvenes. *Nutr Hosp.*, 27, 1334-1343.
 366 Estévez-Santiago, R., Beltrán-de-Miguel, B., Cuadrado-Vives , C. & Olmedilla-Alonso, B.
 367 2013. Software application for the calculation of dietary intake of individual
 368 carotenoids and of its contribution to vitamin A intake. *Nutr Hosp.*, 28, 823-829.
 369 EuroFIR. European Food Information Resource EUROFIR. Available at:
 370 http://www.eurofir.org/?page_id=14 Accessed on September 2015.

371 Fiedor, J. & Burda, K. 2014. Potential Role of Carotenoids as Antioxidants in Human Health
372 and Disease. *Nutrients*, 6, 466-488.

373 Gann, P. H., Ma, J., Giovannucci, E., Willett, W., Sacks, F. M., Hennekens, C. H. &
374 Stampfer, M. J. 1999. Lower prostate cancer risk in men with elevated plasma
375 lycopene levels: Results of a prospective analysis. *Cancer Res*, 59, 1225-1230.

376 George, S. M., Thompson, F. E., Midthune, D., Subar, A. F., Berrigan, D., Schatzkin, A. &
377 Potischman, N. 2012. Strength of the relationships between three self-reported dietary
378 intake instruments and serum carotenoids: the Observing energy and protein Nutrition
379 (OPEN) Study. *Public Health Nutrition*, 15, 1000-1007.

380 Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and cancer: Review of
381 the epidemiologic literature. *Journal of the National Cancer Institute*, 91, 317-331.

382 Granado-Lorencio, F., Olmedilla-Alonso, B., Blanco-Navarro, I., Botella-Romero, F. &
383 Simal-Anton, A. 2006. Assessment of carotenoid status and the relation to glycaemic
384 control in type I diabetics: a follow-up study. *Eur J Clin Nutr* , 60, 1000-1008.

385 Granado, F., Blazquez, S. & Olmedilla, B. 2007. Changes in carotenoid intake from fruit and
386 vegetables in the Spanish population over the period 1964-2004. *Public Health*
387 *Nutrition*, 10, 1018-1023.

388 Granado, F., Olmedilla, B., Bianco, I. & RojasHidalgo, E. 1996. Major fruit and vegetable
389 contributors to the main serum carotenoids in the Spanish diet. *Eur J Clin Nutr* , 50,
390 246-250.

391 Granado, F., Olmedilla, B. & Blanco, I. 2003. Nutritional and clinical relevance of lutein in
392 human health. *Br J Nutr* , 90, 487-502.

393 Granado, F., Olmedilla, B., Blanco, I. & Rojashidalgo, E. 1992. Carotenoid composition in
394 raw and cooked spanish vegetables. *J Agric Food Chem*, 40, 2135-2140.

395 Harper, A. 1999. Defining the essentiality of nutrients. In: Modern Nutrition in Health and
 396 disease (edited by SHILS, M., OLSON, J., SHIKE, M. & ROSS, A.). Pp. 3-10.
 397 Baltimore: Williams and Wilkins.

398 Heber, D. & Bowerman, S. 2001. Applying science to changing dietary patterns. J Nutr, 131,
 399 3078S-3081S.

400 Hoffman, R. & Gerber, M. 2015. Food Processing and the Mediterranean Diet. Nutrients, 7,
 401 7925-7964.

402 Holden, J., Bhagwat, S., Beecher, G., Davis, C., Buzzard, I. M., Eldridge, A., Schakel, S.,
 403 Trainer, D., Gebhardt, S. & Haytowitz, D. 1998. USDA/NCC database for carotenoids
 404 in US foods. Faseb J, 12, A543-A543.

405 Holden, J. M., Eldridge, A. L., Beecher, G. R., Buzzard, I. M., Bhagwat, S., Davis, C. S.,
 406 Douglass, L. W., Gebhardt, S., Haytowitz, D. & Schakel, S. 1999. Carotenoid
 407 Content of U.S. Foods: An Update of the Database. J. Food Comp. Anal., 12, 169-196.

408 Jomova, K. & Valko, M. 2013. Health protective effects of carotenoids and their interactions
 409 with other biological antioxidants. Eur J Med Chem, 70, 102-110.

410 Kant, A. K., Whitley, M. I. & Graubard, B. I. 2015. Away from home meals: associations
 411 with biomarkers of chronic disease and dietary intake in American adults, NHANES
 412 2005-2010. Int J Obes, 39, 820-827.

413 Kijlstra, A., Tian, Y., Kelly, E. R. & Berendschot, T. T. J. M. 2012. Lutein: More than just a
 414 filter for blue light. Progress in Retinal and Eye Research, 31, 303-315.

415 Krinsky, N. I. & Johnson, E. J. 2005. Carotenoid actions and their relation to health and
 416 disease. Mol Aspects Med, 26, 459-516.

417 Liu, R. H. 2013. Health-Promoting Components of Fruits and Vegetables in the Diet.
 418 Advances in Nutrition, 4, 384S-392S.

419 Martinez Burgos, M. A., Martinez-Victoria, I., Mila, R., Farran, A., Farre, R., Ros, G., Yago,
 420 M. D., Audi, N., Santana, C., Lopez Millan, M. B., Lopez, S. R., Manas, M.,
 421 Martinez-Victoria, E. & Network, B. 2009. Building a unified Spanish food database
 422 according to EuroFIR specifications. *Food Chem*, 113, 784-788.

423 Min, J. Y. & Min, K. B. 2014. Serum Lycopene, Lutein and Zeaxanthin, and the Risk of
 424 Alzheimer's Disease Mortality in Older Adults. *Dement Geriatr Cogn Disord*, 37, 246-
 425 256.

426 Moeller, S. M., Volland, R., Tinker, L., Blodi, B. A., Klein, M. L., Gehrs, K. M., Johnson, E.
 427 J., Snodderly, M., Wallace, R. B., Chappell, R. J., Parekh, N., Ritenbaugh, C., Mares,
 428 J. A. & Grp, C. S. 2008. Associations between age-related nuclear cataract and lutein
 429 and zeaxanthin in the diet and serum in the Carotenoids in the Age-Related Eye
 430 Disease Study (CAREDS), an ancillary study of the women's health initiative. *Arch*
 431 *Ophthalmol*, 126, 354-364.

432 Naz, A., Butt, M. S., Sultan, M. T., Qayyum, M. M. N. & Niaz, R. S. 2014. Watermelon
 433 lycopene and allied health claims. *Excli Journal*, 13, 650-666.

434 O'Neill, M. E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., Van den
 435 Berg, H., Hininger, I., Rousell, A. M., Chopra, M., Southon, S. & Thurnham, D. I.
 436 2001. A European carotenoid database to assess carotenoid intakes and its use in a
 437 five-country comparative study. *Br J Nutr* , 85, 499-507.

438 Olmedilla-Alonso, B., Beltran-de-Miguel, B., Estevez-Santiago, R. & Cuadrado-Vives, C.
 439 2014. Markers of lutein and zeaxanthin status in two age groups of men and women:
 440 dietary intake, serum concentrations, lipid profile and macular pigment optical
 441 density. *Nutrition J*, 13.

442 Olmedilla, B., Granado, F. & Rojas-Hidalgo, E. 1993. Quantitation of provitamin and non
 443 provitamin A carotenoids in fruits most frequently consumed in Spain. In: Food and
 444 Cancer Prevention: Chemical and Biological Aspects. Pp. 141-145.

445 Olmedilla, B., Granado, F., Southon, S., Wright, A. J. A., Blanco, I., Gil-Martinez, E., van
 446 den Berg, H., Corridan, B., Roussel, A. M., Chopra, M. & Thurnham, D. I. 2001.
 447 Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from
 448 five European countries. *Br J Nutr* , 85, 227-238.

449 Perveen, R., Suleria, H. A. R., Anjum, F. M., Butt, M. S., Pasha, I. & Ahmad, S. 2015.
 450 Tomato (*Solanum lycopersicum*) Carotenoids and Lycopenes Chemistry; Metabolism,
 451 Absorption, Nutrition, and Allied Health Claims-A Comprehensive Review. *Crit Rev*
 452 *Food Sci Nutr*, 55, 919-929.

453 Rao, A. V., Ray, M. R. & Rao, L. G. 2006. Lycopene. *Adv Food Nutr Res*, **51**, 99-164.

454 Rodriguez Amaya, D. (2001). A guide to carotenoid analysis in foods. OMNI Research, ILSI
 455 Press. Washington, D. C.

456 Seddon, J. M., Ajani, U. A., Sperduto, R. D., Hiller, R., Blair, N., Burton, T. C., Farber, M.
 457 D., Gragoudas, E. S., Haller, J., Miller, D. T., Yannuzzi, L. A. & Willett, W. 1994.
 458 Dietary carotenoids, vitamin-A, vitamin-C, and vitamin-E, and advanced age-related
 459 macular degenerataion. *JAMA*, 272, 1413-1420.

460 Shao, A. & Hathcock, J. N. 2006. Risk assessment for the carotenoids lutein and lycopene.
 461 *Regul Toxicol Pharmacol*, 45, 289-298.

462 Shardell, M., Alley, D., Hick, G. & El-Kamary, S. 2011. Low-serum carotenoid
 463 concentrations and carotenoid interactions predict mortality in US adults: the Third
 464 National Health and Nutrition Examination Survey. *Nutr. Res.*, 31, 178-189.

465 Shashirekha, M. N., Mallikarjuna, S. E. & Rajarathnam, S. 2015. Status of Bioactive
 466 Compounds in Foods, with Focus on Fruits and Vegetables. *Crit Rev Food Sci Nutr*,
 467 55, 1324-1339.
 468 Snodderly, D. M. 1995. Evidence for protection against age-related macular degeneration by
 469 carotenoids and antioxidant vitamins. *Am J Clin Nutr* , 62, 1448-1461.
 470 Stahl, W. & Sies, H. 2003. Antioxidant activity of carotenoids. *Mol Aspects Med* , 24, 345-
 471 351.
 472 Thurnham, D. I. 2007. Macular zeaxanthins and lutein - a review of dietary sources and
 473 bioavailability and some relationships with macular pigment optical density and age-
 474 related macular disease. *Nutr Res Rev*, 20, 163-179.
 475 USDA-ARS, U.S. Department of Agriculture Agricultural Research Service. 2012. What We
 476 Eat in America, NHANES 2009-2010.
 477 USDA, U.S. Department of Agriculture Agricultural Research Service. 2011.
 478 choosemyplate.gov-vegetable-food gallery-Commonly eaten vegetables in each
 479 subgroup.
 480 Vandevijvere, S., Cucu, T., Vinkx, C., Huvaere, K., Huybrechts, I. & Van Loon, J. 2014.
 481 Dietary intake of lycopene by the Belgian adult population. *Public Health Nutrition*,
 482 17, 248-255.
 483 West, C. & Poortvliet, E. 1993. The carotenoid content of foods with special reference to
 484 developing countries. VITAL, International Science and Technology Institute,
 485 Arlington, Virginia.
 486 WCRF, World Cancer Research Fund International. 2007. Food, Nutrition, Physical Activity,
 487 and the Prevention of Cancer: A Global Perspective. Washington DC:, AICR.
 488 WHO, World Health Organization/Food and Agricultural Organization of the United Nations
 489 (WHO/FAO). 2003. Global Strategy on Diet, Physical Activity and Health: Promoting

fruit and vegetable consumption around the world. (edited by ORGANIZATION, W.
H.).

Zampatti, S., Ricci, F., Cusumano, A., Marsella, L. T., Novelli, G. & Giardina, E. 2014.

Review of nutrient actions on age-related macular degeneration. Nutr Res, 34, 95-105.

Table 1. Daily lutein (L), zeaxanthin (Z), lycopene and energy intake from the different food groups in the Spanish adult population

	Total intake (g)	Edible portion (g)	Energy (kcal)	Lutein (µg)	Zeaxanthin (µg)	Lutein-zeaxanthin ^c (µg)	Total L+Z (µg)	L+Z (per 1000 kcal)	Lycopene (µg)	Total carotenoids (µg)	Non-provitamin carotenoids	Non-provit carotenoids /1000 kcal	Provitamin A carotenoids ^d
Milk & dairy products	307.0	306.0	271.0	0.0	0.0	0.0	0.0	0.0	0.0	28.7	0.0	0.0	28.7
Meat & meat products	194.0	175.0	358.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fish & seafood products	89.8	65.9	79.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vegetables	261.1	221.1	86.7	461.2	35.1	280.0	776.4	8954.5	2637.6	4964.8	3414.0	39377.2	1495.0
Fruits	231.0	182.0	130.0	39.1	19.7	5.0	63.8	490.6	329.7	687.9	393.5	3027.0	294.4
Eggs & egg products	31.2	27.1	43.9	0.0	0.0	169.9	169.9	3868.9	0.0	173.4	169.9	3868.9	3.6
Oils & fat	34.4	34.4	304.0	203.9	0.0	0.0	203.9	670.7	0.0	277.7	203.9	670.7	73.9
Beverages	1992	1992.0	169.0	0.0	0.0	20.2	20.2	119.6	37.7	180.6	57.9	342.9	122.6
Aperitifs & appetizers ^a	11.0	10.3	42.6	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0	0.0	5.9
Sauces & herbs & spices	12.7	12.5	22.2	0.0	0.0	1.1	1.1	48.2	50.5	78.0	51.6	2322.6	26.4
Others ^b	227.8	227.8	723.0	n.a ^f	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Total	3392.0	3254.0	2230.0	704.2	54.9	476.1	1235.2 ^e	553.9	3055.6	6397.0	4290.8	1924.1	2106.4

510

511 ^aThe appetizer group includes olives, a salt corn snack (crusts or "rinds"), cocktail crackers, a salted cheese snack (puffs or doodles), popcorn, potato chips, pickles.

512 ^b Grains and grain products, precooked meals, sugars, sweets and baked goods.

513 ^c Lutein-zeaxanthin: dietary content of lutein and zeaxanthin analyzed jointly in certain foods for which separate values for these two carotenoids are not available.

514 ^d Previously published data (Beltran-de-Miguel *et al.*, 2015)

515 ^e Total theoretical intake is 0.0437 mg/kg body weight per day, considering mean body weight to be 70 kg.

516 ^f n.a: no available data.

517

518

519 Table 2. Vegetable and fruit consumption (g/day) in the Spanish adult population and its contribution to daily lutein, zeaxanthin, lycopene and carotenoids intake
520 (µg/day)

Food	Total intake g/d	Non-provitamin A carotenoids µg/d	Lutein µg/d	Zeaxanthin µg/d	Lutein-Zeaxanthin µg/d	Total L+Z µg/d	Total L+Z /g food	Lycopene µg/d	Lycopene /g food
Total diet	3392	4290.8	704.2	54.9	476.1	1235.2	0.4	3055.6	0.9
Vegetables	261.1	3414.0	461.2	35.1	280	776.3	3.0	2637.6	10.1
Artichoke	2.6	2.0	2.0			2.0	0.8	0.0	0.0
Asparagus (wild)	1.8	5.5	5.5			5.5	3.0	0.0	0.0
Broad bean (cooked)	1.2	7.8			7.8	7.8	6.5	0.0	0.0
Broccoli	0.1	1.1	1.1			1.1	10.7	0.0	0.0
Cabbage	1.1	0.6	0.5	0.1		0.6	0.5	0.0	0.0
Carrot (raw and cooked)	10.1	24.8	24.8			24.8	2.5	0.0	0.0
Celery (white, green)	0.7	3.1	3.1			3.1	4.4	0.0	0.0
Corn	2.1	17.1	0.7	0.4	16.1	17.1	8.1	0.0	0.0
Cucumber	5.4	0.6	0.6			0.6	0.1	0.0	0.0
Curly kale	1.1	173.8			173.8	173.8	158.0	0.0	0.0
Eggplant	3.38	25.9			25.9	25.9	7.7	0.0	0.0
Green bean	9.1	30.9	30.9			30.9	3.4	0.0	0.0
Lamb's lettuce	0.3	13.1	13.1			13.1	43.6	0.0	0.0
Leek	1.9	0.5	0.5			0.5	0.3	0.0	0.0
Lettuce	20.7	30.4	30.4			30.4	1.5	0.0	0.0
Onion	23.3	0.4	0.4			0.4	0.0	0.0	0.0
Pepper (red/green)	14.4	41.0	23.3	17.7		41.0	2.8	0.0	0.0
Potato	68.9	8.2	6.1	2.1		8.2	0.1	0.0	0.0
Pumpkin	2.2	10.7	10.7			10.7	4.9	0.0	0.0
Rocket	0.2	16.1	16.1			16.1	80.6	0.0	0.0
Scallion	0.3	0.7			0.7	0.7	2.2	0.0	0.0
Spinach	5.2	182.7	167.8	15.0		182.7	35.1	0.0	0.0

Swiss chard	3	39.5	39.5			39.5	13.2	0.0	0.0
Tomato (fresh tomato sauce)	65.9	2678.2	39.8			39.8	0.6	2638.5	40.0
Turnip greens or tops	0.44	37.1			37.1	37.1	84.4	0.0	0.0
Watercress	0.3	19.9			19.9	19.9	66.4	0.0	0.0
Zucchini	7.1	6.1	6.1			6.1	0.9	0.0	0.0
Vegetable group (individual item: 1-3 g/day)	16.9	205.7	19.6	0.1	186.0	205.7	12.2	0.0	0.0
Vegetable group (individual item intake < 1 g/day)	3.24	88.2	30.4		57.8	88.2	27.2	0.0	0.0
Fruits	231	393.5	39.1	19.7	5.0	63.8	0.3	329.7	1.4
Avocado	1	2.3	2.3			2.3	2.3	0.0	0.0
Apple	41.4	2.0	2.0			2.0	0.0	0.0	0.0
Banana	24.3	1.0	1.0			1.0	0.0	0.0	0.0
Cherry	2	1.0	0.7	0.1		0.8	0.4	0.2	0.1
Grapefruit	0.4	4.1					0.0	4.1	10.3
Kiwifruit	7.1	3.2	3.2			3.2	0.5	0.0	0.0
Orange	34.6	33.4	17.0	16.5		33.4	1.0	0.0	0.0
Papaya	0.9	0.5			0.5	0.5	0.6	0.0	0.0
Peach	15.3	5.0	1.7	3.2	0.1	5.0	0.3	0.0	0.0
Pear	18.3	1.6	1.6			1.6	0.1	0.0	0.0
Persimmon	0.6	5.1			4.3	4.3	7.1	0.8	1.4
Strawberry	2.8	0.4	0.4			0.4	0.1	0.0	0.0
Watermelon	17	329.9	5.3			5.3	0.3	324.6	19.1
Yellow plum	3	2.3	2.3			2.3	0.8	0.0	0.0

521

522 Items that contribute with less than 0.3 µg/day of non-provitamin A carotenoids: a) Vegetables: beet (canned), cauliflower, mushroom, red cabbage, sweet potato;
523 b) Fruits: apricot, blackberry, blueberry, fruit cocktail, grape, lemon, loquat, mango, melon, pineapple, tangerine.

524 Blank space mean no data reported.

525   Table 3. Lutein, zeaxanthin, lycopene and carotenoids intake (µg/day) supplied by fruits and vegetables classified according to color.

526

Fruit and vegetables grouped according to color	Lutein	Zeaxanthin	Lutein-Zeaxanthin	Total L+Z	Lycopene	Total carotenoids	Non-provitamin A carotenoids	% Total non-provitamin A carotenoids intake
White/yellow	225.8	2.4	42.8	271.0	0.0	385.2	271.0	70.4
Red/orange	100.1	37.5	26.1	163.7	2967.3	4806.4	3219.3	67.0
Green	378.3	15.0	237.3	630.6	0.0	993.0	630.6	63.5

527

528

529

530

531

532

533

534

535

536 Table 4. Studies assessing dietary lutein, zeaxanthin and lycopene intakes performed in representative samples of the overall Spanish population.

Year of the survey Sample (N) Spanish general population	Dietary assessment method	FCT used / analytical method	Non-provitamin A carotenoids assessed (µg/p/d)	Reference
1964 (20800 households)	Family Budget surveys	Published food composition data (Olmedilla <i>et al.</i> , 1993, Granado <i>et al.</i> , 1992) / HPLC	Lutein (534.0) Zeaxanthin (108.7) Lycopene (1039.6)	Granado et al 2007
1980 (30311 households)	Family Budget surveys	Published food composition data (Olmedilla <i>et al.</i> , 1993, Granado <i>et al.</i> , 1992) / HPLC	Lutein (676.3) Zeaxanthin (112.5) Lycopene (1414.7)	Granado et al 2007
1990 (21155 households)	Family Budget surveys	Published food composition data (Olmedilla <i>et al.</i> , 1993, Granado <i>et al.</i> , 1992) / HPLC	Lutein (558.0) Zeaxanthin (100.1) Lycopene (1436.6)	Granado et al 2007
2004 (6000 househods)	Food consumption survey	Published food composition data (Olmedilla <i>et al.</i> , 1993, Granado <i>et al.</i> , 1992) / HPLC	Lutein (380.1) Zeaxanthin (73.0) Lycopene (1162.7)	Granado et al 2007

537

538