

Olmedilla-Alonso B, Estévez-Santiago R. Dietary intake of carotenoids: nutritional status assessment and the importance of considering free and esters forms in foods. Capítulo 12, en: Carotenoid esters in foods: Physical, Chemical and Biological Properties. Ed: Adriana Z. Mercadante. Pp. 375-389. The Royal Society of Chemistry (UK), 2019.

Chapter 12

Dietary intake of carotenoids, with emphasis on carotenoid esters

B. Olmedilla-Alonso^{a*} and R. Estévez-Santiago^b

^a Department of Metabolism and Nutrition. Institute of Food Science, Technology and Nutrition (ICTAN-CSIC). C/ José Antonio Novais, 10. 28040-Madrid (Spain).

Phone: +34- 91 549 2300, e-mail: BOlmedilla@ictan.csic.es

^b Área de Ciencia Gastronómica. Facultad de Ciencias Jurídicas y Empresariales. Universidad Francisco de Vitoria (UFV). Carretera Pozuelo-Majadahonda km. 1,800 28223 Pozuelo de Alarcón-Madrid (Spain). Phone: +34 91 351 0303, e-mail: rocio.estevez@ufv.es

* Corresponding contributor. E-mail: BOlmedilla@ictan.csic.es

Abstract

Carotenoids display biological activities relevant to human health and thus, it is of great interest to evaluate their nutritional status, which can be carried out using different and complementary approaches, such as the dietary intake assessment. This chapter focuses on the dietary intake of the major carotenoids found in the human diet and on dietary methods for their assessment. In the evaluation of food consumption, special attention should be made to fruit and vegetable intake. In food conversion into the carotenoid intake, it is essential to provide the correct food identification and the food intake estimate, as well as the food composition tables used. In most fruits and some vegetables, the main xanthophylls present in the human diet (lutein, zeaxanthin, β -cryptoxanthin) are found in both free form and esterified with fatty acids. Although there is very few information on those ester forms, dietary xanthophylls are ingested in quite a higher percentage as ester forms, for which a higher bioavailability and a higher conversion to retinol in the case of β -cryptoxanthin have been described. Finally, the adequacy of carotenoid intake is discussed in relation to the contribution of the vitamin A intake and, in terms of the effects of individual carotenoids.

12.1 Introduction

The extensive investigation on food and nutrition in relation to health and disease carried out during the 20th century led to the identification of diverse bioactive components of the diet as factors implicated in the prevention and in reducing the risk of a number of chronic diseases. These include carotenoids, fat-soluble pigments widely present in the diet. In humans, carotenoids are essential precursors for the production of retinoids, such as vitamin A. In addition, some carotenoids display other biological activities relevant to human health. In fact, a high intake of carotenoids is associated with an enhancement of the immune system and cognitive function and a lower risk of development (or progression) of several chronic diseases, such as age-related macular degeneration, type 2 diabetes, cardiovascular diseases and some types of cancer, among others.¹

The diet is one of the main factors, related to lifestyle, through which we can influence the morbidity and mortality associated with diverse diseases. Thus, the evaluation of the nutritional status is an essential aspect in improving health at the level of individuals and in populations, and this can be carried out using different and complementary approaches, including anthropometry data, the biomarkers of exposure and of intake effect, the clinical examination of signs caused by an imbalanced nutrient intake, and dietary intake assessment. In the selection of the method for nutritional evaluation, it is indispensable to consider the setting (clinical practice, public health or research) in which the results are going to be employed since each has advantages and inconveniences with respect to its utilization in individuals or populations.^{2,3}

Carotenoid nutritional status is assessed using dietary or biochemical methods, and the serum concentration has been considered the best method for establishing their nutritional status in humans.⁴ In the dietary intake and in human blood, only six carotenoids are usually determined, three carotenes (β -carotene, α -carotene, lycopene) and three xanthophylls (lutein, zeaxanthin and β -cryptoxanthin),⁵ although about 50 carotenoids can be present in the human diet,⁶ and there are an increasing knowledge and interest in the biological activities of other carotenoids such as neoxanthin, violaxanthin, phytoene and phytofluene. Further and updated information can be found in two recent reviews on metabolism, biotechnology, and nutritional and health benefits¹ and on a database of carotenoid content in foods.⁷

To assess carotenoid status, special attention must be given to the intake of fruits and vegetables, which are the major contributors to their dietary intake. However, the contribution of animal-derived food should not be overlooked,⁶ as eggs, dairy products and seafoods contain significant concentrations of carotenoids, mainly lutein, zeaxanthin, β -carotene and astaxanthin

(see Chapter 7). Moreover, carotenoids are also present as additives and can be ingested as food supplements. This chapter focuses on the dietary intake of the abovementioned major carotenoids found in the diet and human serum and on dietary methods for their assessment.

Most fruits and vegetables contain β -carotene and lutein. β -Cryptoxanthin is usually found in low concentrations in fruits but it is a major carotenoid in ripe red and orange plant sources. Lycopene is present in a few primary sources (mainly tomato, watermelon and pink grapefruit), but is widely distributed in processed foods, as a tomato-based product.⁶ In most fruits and some vegetables, the main xanthophylls present in the human diet (lutein, zeaxanthin, β -cryptoxanthin) are found in both free form and esterified with fatty acids.^{6,8} However, the presence of carotenoid esters has often been overlooked, mainly due to the use of saponification as a routine step in the carotenoid analysis that can provoke destruction or structural transformation of the carotenoids (see Chapter 8).⁸⁻¹⁰ When this procedure is not thoroughly assessed and optimized, the carotenoid food content could be underestimated¹¹ and, eventually, it would contribute to an underestimation of the carotenoid dietary intake.

12.2 Assessment of Carotenoid Dietary Intake

In a dietary assessment of carotenoids, the first step is the evaluation of food consumption and, the second, the conversion of food into the nutrient intake. We need to know the amount ingested (the weight of the portion consumed or of the usual average portion, depending on the population or household servings). We have to have access to food composition tables (FCT) or food composition databases (FCDB), that provide data on the content of the compound or compounds that are the objects of the study.^{12,13} Food intake can be studied using a wide variety of methods and the selection is influenced by the type of sample (individuals or population), the design and the objective of the study, as well as the available resources, among others. In any case, the method must be validated to confirm that the researchers measured what they intended to measure.

Carotenoid current intake is generally assessed by means of two approaches, the weight of the food (the most accurate procedure) or by means of the estimate of the food consumed (the difference with respect to the previous approach is that the amount consumed is estimated utilizing household portions or photos of the food portions and plates that are habitually consumed). The techniques, that measure habitual intake, obtain the information by means of a) 24-hour dietary recalls, b) dietary records, c) dietary history, d) food frequency questionnaire. The main inconvenience of these methods is that they are based on data in the memory of the person surveyed, an aspect that can be attenuated, in the case of it not being self-reported, with

the ability of the interviewer. The evaluation of the habitual intake is widely utilized for nutritional epidemiology since diet is one of the (modifiable) factors implicated in a number of diseases.

12.2. 1 Assessment Methods used in Populations and Individuals.

The dietary intake assessment at the level of a general population can be done directly using food intake surveys at a national level and, in Europe, should be conducted according to the recommendations of the last European Food Safety Authority (EFSA)¹⁴ in representative population groups. However, indirect assessment is a more frequent approach in the general population and, the unit of study is the household, not the individual. The dietary intake of the population is assessed by a) food balance sheets, b) household budget surveys, c) specific consumption surveys.¹⁵

Food balance sheets are frequently used and consider the consumption per capita, obtained by dividing the total annual amounts of each food by the population in each country and in the year studied (kg/capita/year or g/person/day), assuming a constant consumption throughout the year. Food classification is of great importance and codification systems, such as Languel or FoodEx2,¹⁶ should be used. Household budget surveys are periodically used to assess consumption in households utilizing diverse methods, such as daily records (generally over 7 days), the recalls of a food list, inventory and recount.

Surveys and questionnaires are utilized for the assessment of individual intake. In both cases, it is preferable that they are carried out by a trained interviewer, especially the surveys. When dietary records are utilized, the individual will need to receive extremely detailed instructions, especially in relation to the main sources of the dietary components under study. This method consists in requesting interviewees taken down daily for 3, 5, 7 or more days the foods and beverages they ingest, as well as their amount. In the case of a record of 3 days, it should be carried out during a single week and include one day of the weekend. Dietary recall consists in remembering and taking down all the foods and beverages consumed, as well as the form in which the plates are prepared, over the last 24 hours. It is one of the techniques most widely used because it is simple to do and, in general, to achieve the best reliability of the information. It is necessary to perform 24-hour recalls on three days, one of which should correspond to a holiday or weekend day.

To assess intake by means of food frequency questionnaire (FFQ), it is necessary to utilize list of foods about which the interviewee should respond in terms of the daily, weekly or monthly consumption of each food over the past month, or 6 or 12 months. Attention must

be made not to overestimate or underestimate consumption. The questionnaire can be closed (including questions about foods that are of interest in a specific study) or open (the individual can include the foods consumed). The preparation of this type of questionnaire is specific for each study and depends on the type of components of the foods that should be assessed. The total energy provided by the diet is needed to express the carotenoid intake concentrations in terms of dietary energy density (each carotenoid/1000 kcal), which enables a more reliable comparison of results across studies. There is no standard FFQ and, in the case of carotenoids, it is highly important to include a detailed and complete list of foods of vegetable origin, as well as to note that foods that have a high carotenoid content does not always classify a given food as a good contributor to dietary carotenoid intake; the importance is also related to the frequency of consumption. FFQs and 3-24h records are methods widely used in the assessment of dietary carotenoids intake and correlations between the results and serum carotenoid concentrations can be found in the literature, where for instance, higher coefficients were obtained using semiquantitative FFQ for all carotenoids, except β -cryptoxanthin.¹⁷

Finally, another procedure for the evaluation of intake is the diet history questionnaire (DHQ). This utilizes 24-hour recalls, food frequency questionnaires and additional questions related to the aim of each study.

Recording the diet for several days (3-7 days) and 24-hour recalls are the most frequently utilized forms for assessing ingestion in individuals; they are rapid, have a low financial cost and need only simple training on the part of the interviewer. The main disadvantage of the day-recording is that it usually changes the pattern of feeding on the part of the subject, and that of 24-hour recall is that it depends on the memory of those being surveyed. On the other hand, in epidemiological studies, food-frequency questionnaires are one of the key research tools, but their usefulness may be limited when they are poorly designed and used inappropriately, since they may not yield the required information.¹⁸

12.2.2 Dietary Questionnaires

The questionnaires utilized in studies should enable the collection of information that can be quantifiable, verifiable and comparable.¹⁸ In the design and development of a questionnaire on ingestion, the object of the study must be clearly established, as should the specific objectives, the population and sample being studied, the technique for collecting the information and, ultimately, the questionnaire must be validated. Questionnaires can be designed specifically for a study, or previous questionnaires can be utilized, once they have been adapted to the necessities of the study.

In the preparation of the questionnaire, the questions must be clear and worded in a way that does not influence the response; in addition they should define what components of the diet are to be assessed, in what chemical forms they can be presented (e.g. free carotenoids, in ester form, total forms or isomers) and in what type of food are they encountered.

In general, in all questionnaires for diet assessment, it is useful to include other data of general interest that will facilitate a proper interpretation of the results (e.g., age, body weight and height), and it is preferable that they made be measured *in situ*, rather than being self-reported. In the case of carotenoids, it is also interesting to know those factors that may influence absorption and metabolism of the carotenoids, for example, use of tobacco, chronic disease, use of medication or food supplements.

Before generalizing the application of a questionnaire, it is necessary to evaluate its validity and reliability, which will enable the comparability of the results. Validity (accuracy) and reliability (reproducibility) are highly influenced by the characteristics of the questionnaire design. Reliability (or precision) is a measure of the ability of the questionnaire to distinguish to what degree does a variable fluctuate as the result of an error in measuring or as the result of an actual change. Reliability is influenced both by the type of sample and the number of foods/components that are being assessed, as well as the estimate and quantification of the portions/food helpings. Validity refers to the capacity of a procedure/method or instrument to really measure what we expect to measure and determines the level of confidence that can have in the inferences that we obtain from the results. The validity of a questionnaire to estimate similar intakes to those obtained using other methods is studied by comparing the results with those obtained by other methods considered a reference (e.g., with dietary records, with 24-hour recalls). The correlation between the two should be between 0.4 and 0.8; less than 0.4 implies that the reliability of one of them is unacceptable or that both of them are measuring different things. The validity of the estimation of food ingestion is frequently associated with factors, such as sex, age, etc.; thus, personal characteristics must be taken into account in the design of questionnaires.

The validation of dietary questionnaires is usually done with another measure of dietary intake (*i.e.* the FFQ with multiple recalls or records as the reference instrument, the diet history questionnaire with three or four 24-hour dietary recalls) and with serologic markers of diet as the standard. For instance, in The Eating at America's Table Study (EATS), the validity coefficients between carotenoid concentration in serum and in the diet (DHQ and multiple 24-hour recalls) were higher for α -carotene, β -carotene and β -cryptoxanthin than for lycopene and lutein. DHQ was comparable to other FFQs for use in large epidemiological studies. In this and

other studies, the variability in the serum–diet correlations due to the use of methods/procedures greatly depends on the population group, their particular dietary intakes, the proximity of blood collection to administration of the four 24-hour recalls, and other unknown factors, thus, the combination of two procedures may provide better estimates of usual intake than using either procedure alone.¹⁹

Finally, the application of technological advances in methods of collecting dietary intake data, with increased use and access of the internet and smartphones, needs to be considered as there is a tendency to use self-administered methods rather than interviewer administered or paper-based surveys and thus, the assessment of their accuracy is needed. It was the aim of a recently published systematic review, to evaluate the accuracy of technology-based dietary assessment methods (most of them retrospective measures of diet) to determine carotenoid and/or fruit and vegetable intake when compared with carotenoid biomarkers. Moderate correlations were found, but some of those methods provide good estimates of carotenoid intake.²⁰

12.2.3 Food Intake and Measurement of Carotenoid Intake

The food ingested must be correctly identified, *i.e.* using databases on food consumption (e.g. Langual, EuroFIR) and transformed into grams of ingested food using tables of standard helpings, household portions or recipes. (e.g.¹³)

For the transformation of grams of food/day in μg carotenoids/day, food composition tables or databases (FCT/FCDB) are used, including individual data on carotenoids.^{7,21-24} There are diverse factors that influence the composition and content of carotenoids in foods. They include the geographic region and the climate, seasonality, growth conditions, among others,^{1,25} therefore, it is preferable the utilization of the FCT that contains data on the food of the country in which the assessment of carotenoid ingestion is being carried out, provided it meets minimum quality criteria. However, in Europe, the FCTs, constructed according to European standards and available in the EuroFIR platform using the FoodExplorer tool, do not supply data of individualized carotenoid food content. For instance, one of those FCTs is the Spanish Food Composition Database (BEDCA, for its Spanish name)²⁶⁻²⁸ which do not supply data on individual carotenoids, although there are many studies published on these compounds in Spanish foods.^{24,29,30}

Traditionally, FCT/FCDB only include data on β -carotene, because of its provitamin A activity, providing its concentration or just taking into account its contribution to the vitamin A food content. Data on the individual major dietary carotenoids are limited in FCT, among them

are those of West and Poortvliet (1993),²¹ those of the USDA,^{22,31} and the recently published database of carotenoid contents in Iberian-American foods, that includes data generated exclusively by high-performance liquid chromatography (HPLC), isomeric forms *E* and *Z*, and detailed information on the procedure of the analysis and description of the food.⁷

The level of confidence in FCT of a user is determined by diverse aspects that influence the variability of the data, which includes whether the sample is representative, the identification of the food, the analytical method and the term used to refer to food carotenoid content (*i.e.* total carotenoid content, carotene). All of these aspects can mean that the results are not comparable and that there is an overestimation or underestimation of the content.^{7,10,32} Thus, the utilization of a single source can magnify the error, depending on the frequency of consumption of certain foods and, as a result, the “true” ingestion in a population or group may be disguised, weakening the power of the study and leading to erroneous conclusions.¹⁰

The identification of the food includes the following aspects: local name and scientific name, description of the food, part of the plant consumed and percentage of the comestible portion, analysis of the food when raw, cooked or processed, the degree of ripening, seasonality and humidity of the sample. With respect to the analytical method, information not usually reported in the FCTs, HPLC is the preferred approach for the analysis of carotenoids in the different food matrixes.⁷ An important aspect of the analysis is carotenoid extraction, in which saponification is a routine step with the aim of removing chlorophylls and lipids to release a clean preparation for analysis (see Chapter 8). Thus, the presence of carotenoid esters has often been overlooked and there is nearly no information on xanthophylls esters in the literature, although they are of great interest as they have more stability during food processing and facilitate the solubilization and extraction (bioaccessibility) during digestion³³, and also because their bioavailability is equivalent or even higher than that of free carotenoids (see Chapter 13).³⁴ In fact, saponification can also provoke destruction or structural transformation of the carotenoids, to a greater or lesser degree, according to the carotenoid and the food type,^{8-10,33} and to avoid or minimize losses of carotenoid during saponification, this procedure should be thoroughly assessed and optimized. Otherwise the carotenoid content could be underestimated in the food¹¹ and, eventually, it would contribute to an underestimation of carotenoids intake.

Finally, FCT does not include data on the bioavailability of carotenoids, that notify about the compound amount in the food that is absorbed by the gut and available for use or storage by the body.³⁵ However, this is an aspect of great importance to establish effective vitamin A intervention programs and dietary recommendations to reduce the risk of certain chronic disease in population groups (*i.e.* lutein and zeaxanthin in subjects at risk for age-related

macular degeneration). Although bioavailability varies depending on the type of carotenoid and food matrix,^{1,25,36,37} the contribution of provitamin A carotenoids to the dietary intake of vitamin A is calculated assuming equal bioavailabilities of α -carotene and β -cryptoxanthin and that both contribute in the same proportion to vitamin A intake, each being half the bioconversion factor of β -carotene in the formulas used to express vitamin A activity: Retinol equivalents (RE) ($\mu\text{g}/\text{day}$) = retinol + (β -carotene/6) + (α -carotene/12) + (β -cryptoxanthin/12)^{13,38} or as retinol activity equivalents (RAE) ($\mu\text{g}/\text{day}$) = retinol + (β -carotene/12) + (α -carotene/24) + (β -cryptoxanthin/24).³⁹ However, β -cryptoxanthin, the most important provitamin A xanthophyll in the human diet and found mainly in ester forms in red/orange coloured fruits, seems to be more efficiently absorbed and converted into retinol than the carotenes.⁴⁰⁻⁴² To date, this and other xanthophyll esters (mainly, lutein and zeaxanthin) seem to have equal or higher bioavailability than their free forms.^{8,33}

12.3. Carotenoid Dietary Intakes

Dietary carotenoid intake varies greatly among subjects and within subjects, as well as among populations, derived from natural or intrinsic factors (*i.e.* dietary changes, seasonality) or methodological aspects (*i.e.* methods of assessing ingestion and reliability of the FCT).^{1,7,10,39} Thus, the comparability of the ingestion of carotenoids among population-based studies is influenced by aspects such as the degree of representativeness of the sample (*i.e.* sample size, age ranges, gender), the types of dietary questionnaires and the FCT used.^{25,43,44}

The individual carotenoid intake in Europeans has been assessed in few reports, although there are many studies in which β -carotene is particularly assessed. The dietary carotenoid intake from a selection of studies has been included in a recent review.¹ A great variability in the dietary intake data was observed in groups of populations from Europe and the USA, as individual carotenoids (mg/day, mean intake) the ranges were around: 1.7 and 8.8 for β -carotene, 0.2 and 2.4 for α -carotene, 1 and 4 for lutein+zeaxanthin, 1.6 and 8.1 for lycopene and 0.3 and 1.4 for β -cryptoxanthin, respectively.

There are many studies on the dietary intake of the main carotenoids in groups of subjects, but very few data in representative samples of populations provided by national surveys, *e.g.* USA, Spain, Brazil.⁴⁴⁻⁴⁶ However, those could be compared given the fact that several variables are considered, such as age and sex, the dietary assessment method and the analytical method used in the data provided by the FCT. In fact, individual dietary carotenoid intake in the USA and Spain (from the whole diet)⁴³⁻⁴⁵ and in Spain and Brazil (from fruit and vegetable intake)^{43,44,46} were compared and the discrepancies and similarities discussed in a

recent article on the dietary intake in the Brazilian population.⁴⁶ In addition, data on dietary phytoene and phytofluene intake in the population of Luxemburg,⁴⁷ as well as that of neoxanthin and violaxanthin in the Brazilian population⁴⁶ have been reported.

The carotenoid intakes in populations from Spain, the United States and Brazil, using data on food consumption obtained from Dietary Intake Surveys,⁴³⁻⁴⁶ are shown in Table 12.1. These intakes were obtained from representative samples of the population during the same or similar periods of time and using FCT containing carotenoid data generated by HPLC. However, there are differences that could affect the results obtained, such as the range of age of the populations (Spain: 18 - 64 years, Brazil: 10 years and over, USA: 20 years and over), the food items considered (*i.e.* only raw fruits and vegetables or a large number of processed foods, included when whole diet is assessed).

[Table 12.1 near here]

In an attempt to evaluate to what extent xanthophylls are ingested as free or in ester forms, we utilized data on food consumption in the Spanish and Brazilian populations (previously employed to assess the ingestion of carotenoids and of their major dietary sources),^{43,44,46} and the data on the content of xanthophylls in ester forms and in free forms in fruits given in Chapter 7 of this book. Among those fruits, we selected those that are main sources of xanthophylls in Brazil and Spain: apple, orange, papaya, peach, pepper, persimmon, tangerine and mango, All of them are fruits and contain more xanthophyll ester forms than vegetables do.^{8,48} They are mainly sources of β -cryptoxanthin, more than sources of lutein and zeaxanthin, since, from them, only orange, pepper and mango are good contributors to lutein and zeaxanthin intakes, . The ingestion of xanthophylls in ester form represents approximately 70% of the total dietary xanthophylls intake in both the Spanish and Brazilian populations. This value was calculated using mean estimates approximate to the data on the concentration expressed as mean or median (not specified) or as a range (we did not consider the data expressed as percentages). The xanthophylls content ($\mu\text{g/g}$, fresh weight) in the abovementioned foods, as free and ester forms, was approximately the following: apple (free: 0.4; esters: 2.1), orange (free: 33; esters: 81), papaya (free: 88; esters: 261), peach (free: 1.5; esters: 1.6), pepper (red/green) (free: 122; esters: 172), persimmon (free: 0.9; esters: 12.4), tangerine (free: 3.9-2.6; esters: 40-88.5), mango (free: 30.4; esters: 33.3). Based on the data from the abovementioned limited number of foods, the consumption of xanthophylls, in free and ester forms, in each country would be approximately 35% and 65%, respectively, in the

Spanish population, and 28% and 72%, respectively in the Brazilian population. Based on data on fruit and vegetable intake (g/person/day) in those populations,^{44,46} the approximate consumption of free and ester forms of xanthophylls, from each of those foods, is shown in **Table 12.2.**

[Table 12.2 near here]

12.4 Interpretation of Dietary Carotenoid Intake

The adequacy of carotenoid intake can be established in relation to the contribution of the vitamin A intake of those carotenoids having provitamin A activity and in terms of the effects of individual carotenoids. The intake of every carotenoid has been associated with a lower risk of several chronic diseases (*i.e.* lutein and zeaxanthin with some ocular diseases, lycopene with prostate cancer, β -cryptoxanthin with an increase in bone mass) and each one of the six main carotenoids in human serum and diet has different effects in relation to the causes of mortality and, moreover, in relation with the interactions among them.⁴⁹ However, so far, there is no recommendation concerning dietary intake of individual carotenoids, and thus, the adequacy of carotenoid intake could be assessed in terms of the concentrations related to health effects or that achieve risk reduction for certain chronic diseases (*e.g.* age-related macular disease) reported by epidemiological studies.

In general, the adequacy of the intake is referred to the dietary intake recommendations established by each country or adapted to the specific characteristics of the population. They are established only for nutrients, but not for other bioactive compounds of the diet. The dietary reference intake (DRI) provides nutrient reference values developed by the Institute of Medicine of The National Academies of the USA,³⁹ that are widely adopted around the world (for instance, for vitamin A: 900 μ g and 700 μ g RAE/day, for men and women, respectively). In the establishment of the DRI, it is taken into account the possible losses that can take place, both for treatment of the food (culinary, industrial, preservation, etc.) and for the incomplete utilization due to individual variability in cases of digestion, absorption and metabolism. However, the increasing knowledge in bioavailability and bioconversion of the major dietary provitamin A carotenoids (β -carotene, α -carotene and β -cryptoxanthin) has not been taken into account and, thus, the assessment of the contribution of provitamin A carotenoids to the recommended dietary intake of vitamin A should be revised. On the basis of recent studies, the contribution of carotenoids to dietary vitamin A expressed as RAE is being questioned, since

those of β -cryptoxanthin and α -carotene would be greater than those currently assigned and, on the other hand, β -cryptoxanthin, supplied in the diet as an ester form, seems to have a greater bioavailability than β -carotene.^{36,40,41} Moreover, the effect of the diverse polymorphisms in their conversion to vitamin A should be considered.^{1,25,41} Provitamin A carotenoids are present in different types of food matrices, physical forms (e.g. crystalline, amorphous) and chemical structures (carotene *vs* xanthophyll) and, on the other hand, hydrolysis of xanthophylls (mono and diesters) varies during the digestion depending on the type of xanthophyll (regarding provitamin A, only α - and β -cryptoxanthin can be considered) in a given food as well as for a given xanthophyll in different foods.^{8,10}

On the other hand, there is increasing agreement on the need for a dietary reference intake-like recommendations for lutein, as this carotenoid meets the criteria for a non-essential bioactive substance regarding the promotion of optimal health and/or prevention of chronic diseases.^{50,51} On the basis of the extensive information currently available on its content in foods and in serum, and in human intervention studies (with diverse objectives), among others, a serum lutein concentration in the range 34 - 60 $\mu\text{g lutein dl}^{-1}$ seems to be desirable⁴ to achieve benefits in ocular health and ensure an optimal availability to the tissues, since those levels have been associated with beneficial effects, with no risk of possible secondary effects (the level of ingestion considered safe is 20 mg day^{-1}).⁵² This concentration in blood can be reached through a mean ingestion of 6 mg of lutein and zeaxanthin per day, by means of a habitual ingestion of foods rich in lutein. This makes it possible to ensure a somewhat higher contribution than that mentioned above since the bioavailability from foods and from chemical forms (*i.e.* free *vs* ester) is variable.^{1,25,33,36,37}

12.5 Conclusions

Dietary carotenoid intake assessment would be more accurate if FCT with data on individualized carotenoid content (generated by HPLC in foods from the country in which the assessment is done) is utilized, along with information on the analytical methods and, when possible, bioavailability data from different types of food matrixes and carotenoids forms (*i.e.* free *vs* ester forms, *E vs Z* isomers). This procedure would enable a better comparison of results across studies.

Given the high percentage in which the diet supplies xanthophylls in ester form (two or three-fold higher than the free form), the different bioavailability of xanthophylls in free or ester forms,^{8,34,37} as well as the highest conversion to retinol, in the case of β -cryptoxanthin,^{36,40-42} it is necessary to perform an in-depth study of the ester forms present in the diet, to achieve a

better comprehension of the existing data on dietary carotenoid intakes and to design more reliable dietary recommendations in relation to the different health objectives being pursued.

References

1. M. Rodríguez-Concepción, J. Avalos, M.L. Bonet, A. Boronat, L. Gomez-Gomez. D. Hornero-Méndez. M. C. Limón, A.J. Meléndez-Martínez, B. Olmedilla-Alonso, A. Palou, J. Ribot, M.J. Rodrigo, L. Zacarias, C. Zhu, *Prog. Lipid Res.*, 2018, **70**, 62.
2. R.E. Patterson, P. Pietinen, *Nutrición y Salud Pública*, ed. M. L. Gibney, B. M. Margetts, J. M. Kearney, L. Arab, Blackwell Science Ltd, Oxford, UK, and Ed. Acribia, S.A. (Zaragoza, España), 2006, **3**,73-91.
3. B. Olmedilla Alonso, *Alimentos funcionales: importancia del laboratorio clínico y nuevas perspectivas*. Ed. B. Olmedilla. Comité de Comunicación de la Sociedad Española de Química Bioquímica Clínica y Patología Molecular, Barcelona, 2010, **1**, 11-29.
4. F. Granado, B. Olmedilla, I. Blanco, *Br. J. Nutr.*, 2003, **90**, 487.
5. A.V. Rao, L.G. Rao, *Pharmacological Research*, 2007, **55**, 207.
6. G. Britton, F. Khachik, *Carotenoids*, ed. G. Britton, S. Liaaen-Hensen, H. Pfander, Birkhauser, Basel, Switzerland, 2009, **5**,3 45-66.
7. M. G. Dias, B. Olmedilla-Alonso, D. Hornero-Méndez, A.Z. Mercadante, C. Osorio, L. Vargas-Murga, A.J. Meléndez-Martínez, *J. Agric. Food Chem.*, 2018, **66**, 5055.
8. A. Z. Mercadante, D. B. Rodrigues, F. C. Petry, L. R. B. Mariutti, *Food Res. Int.*, 2017, **99**, 830.
9. K.T. Amorim-Carrilho, A. Cepeda, C. Fente, P. Regal, *Trends Anal. Chem.*, 2014, **56**, 49.
10. F. Granado, B. Olmedilla, I. Blanco, E. Gil-Martínez, E. Rojas-Hidalgo, *Crit. Rev. Food Sci. Nutr.*, 1997, **37**, 621.
11. D.B. Rodríguez-Amaya. Food Carotenoids: Chemistry, Biology and Technology. *Institute of Food Technologists, IFT Press series*, Chicago, USA, 2016.
12. E. Martínez-Victoria, I. Martínez de Victoria, M. A. Martínez-Burgos. *Nutr Hosp.* 2015, **31**(3), 168.
13. O. Moreiras, A. Carbajal, M.L. Cabrera, C. Cuadrado, *Tablas de Composición de Alimentos. Guía de Prácticas*. Anaya, Ediciones Pirámide, Madrid, 2013, 401-412.
14. EFSA (European Food Safety Authority), *Guidance on the EU Menu Methodology*, Parma, Italy, 2014.
15. J.M. Martín-Moreno, L. Gorgojo, *Rev. Española de Salud Pública*, 2007, **81**, 507.

16. EFSA Technical Report, The food classification and description system FoodEx2 (rev. 2). 2015. <https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2015.EN-804>
17. F. Granado-Lorencio, B. Olmedilla-Alonso, I. Blanco-Navarro, F. Botella-Romero, A. Simal-Antón. *Eur. J. Clin. Nutr.*, 2006, **60**, 1000.
18. J.E. Cade, B.J. Burley, D.L. Warm, R.L. Thomson, B.M. Margetts, *Nutr. Res. Rev.*, 2004, **17**, 5.
19. L.B. Dixon, A.F. Subar, L. Wideroff, F.E. Thompson, L.L. Kahle, N. Potischman, *J. Nutr.*, 2006, **136**, 3954.
20. T.L. Burrows, M.E. Rollo, R. Williams, L.G. Wood, M.L. Garg, M. Jensen, C.E. Collins. *Nutrients*, 2017, **9**, 140.
21. C.E. West, E.J. Poortvliet, *The carotenoid content of foods with special reference to developing countries*. Ed: Vitamin A Field Support Project (VITAL), International Science and Technology Institute, Inc., Virginia (USA), 1993, 1-207.
22. USDA-NCC Carotenoid Database for U.S. Foods. 1998. Agricultural research service, U.S. Department of Agriculture. http://webapp1.dlib.indiana.edu/virtual_disk_library/index.cgi/4298263/FID974/ndldata/car98/car_ref.pdf , accessed 07/19, 2018.
23. M. O'Neill, Y. Carroll, B. Corridan, B. Olmedilla, F. Granado, I. Blanco, *Br. J. Nutr.*, 2001, **85**, 499.
24. B. Beltrán, R. Estévez, C. Cuadrado, S. Jiménez, B. Olmedilla Alonso, *Nutr. Hosp.*, 2012, **27**, 1334.
25. G. Maiani, M.J. Periago-Castón, G. Catasta, E. Toti, I. Goñi-Cambrodón, A. Bysted, F. Granado-Lorencio, B. Olmedilla-Alonso, P. Knuthsen, M. Valoti, V. Böhm, E. Mayer-Miebach, D. Behnlian, U. Schlemmer, *Mol. Nutr. Food Res.*, 2009, **53**, S194.
26. M.A. Martínez Burgos, I. Martínez-Victoria, R. Milá, A. Farrán, R. Farré, G. Ros, M.D. Yago, N. Audi, C. Santana, M.B. López Millán, S. Ramos López, M. Mañas, E. Martínez-Victoria, and on behalf of network BDECA. *Food Chem.* 2009, **113**, 784.
27. G. Ros, E. Martínez de Victoria, A. Farran, *Food Chem.*, 2009, **113**, 789.
28. BEDCA - Base Española de datos de composición de alimentos. Agencia Española de Seguridad Alimentaria y Nutrición, 2006. http://www.bedca.net/http://www.aecosan.msssi.gob.es/AECOSAN/web/seguridad_alimentaria/subseccion/composicion_alimentos_BD.htm, accessed 07/19, 2018.

29. B. Olmedilla, F. Granado, I. Blanco, E. Rojas-Hidalgo, *Food and Cancer Prevention: Chemical and Biological Aspects*, ed. K. W. Waldron, I. T. Johnson, G. R. Fenwick, Royal Society of Chemistry Special Publications. Cambridge: Royal Soc. Chemistry, 1993, 141-145.
30. F. Granado, B. Olmedilla, I. Blanco, E. Rojas-Hidalgo, *J. Agric. Food Chem.*, 1992, **40**, 2135.
31. J. Holden, A. Eldrige, G. Beecher, I. Buzzard, S. Bhagwat, C. Davis, *J. Food Comp. Anal.*, 1999, **12**, 169.
32. M. Faber, F.A.M. Wenhhold, U.E. MacIntyre, E. Wentzel-Viljoen, N.P. Steyn, W.H. Oldewage-Theron, *Nutrition*, 2013, **29**, 1286.
33. A. Bunea, C. Socaciu, A. Pinte. *Not. Bot. Horti Agrobi.*, 2014, **42**, 310.
34. L. R. B. Mariutti, A.Z. Mercadante. *Arch. Biochem. Biophys.*, 2018, **648**, 36.
35. P.J. Aggett, *Am. J. Clin. Nutr.*, 2010, **91**, 1433S.
36. Tang, G. *Am. J. Clin. Nutr.*, 2010, **91**, 1468S.
37. F. Granado-Lorencio, B. Olmedilla-Alonso, C. Herrero-Barbudo, B. Pérez-Sacristán, I. Blanco-Navarro, S. Blázquez-García, *J. Agric. Food Chem.*, 2007, **55**, 6387.
38. FAO/WHO. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation. FAO, Rome, 2001.
39. Institute of Medicine. *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*. National Academy Press. Washington DC, 2000.
40. R. Estévez-Santiago, B. Olmedilla-Alonso, I. Fernández-Jalao, *Food Funct.*, 2016,**7**,1354.
41. B.J. Burri, J.S.T. Chang, T.R. Neidlinger, *Brit. J. Nutr.*, 2011, **105**, 212.
42. S. de Pee, C.E. West, D. Permaesih, S. Martuti, Muhilal, J.G. Hautvast. *Am. J. Clin. Nutr.*, 1998, **68**, 1058.
43. R. Estévez-Santiago, B. Beltrán-de-Miguel, B. Olmedilla-Alonso. *Int. J. Food Sci, Nutr.*, 2016, **67**, 305.
44. B. Beltrán-de-Miguel, R. Estévez-Santiago, B. Olmedilla-Alonso. *Int. J. Food Sci. Nutr.*, 2015, **66**, 706.
45. NHANES, 2009–2010. What We Eat in America.
https://www.ars.usda.gov/ARUserFiles/80400530/Pdf/0910/Table_1_Nin_Gen_09.Pdf
https://www.ars.usda.gov/ARUserFiles/80400530/pdf/0910/Table_37_SUP_GEN_09.pdf
46. L. Vargas-Murga, V.V. de Rosso, A.Z. Mercadante, B. Olmedilla-Alonso, *J. Food Comp. Anal.*, 2016, **50**, 88.

47. E. Biehler, A. Alkerwi, L. Hoffmann, E. Krause, M. Guillaume, M.L. Lair, T. Bohn. *J. Food Comp. Anal.*, 2012, **25**, 56.
48. R. Estévez-Santiago, B. Olmedilla-Alonso, B. Beltrán-de-Miguel, C. Cuadrado-Vives, *Nutr. Res.*, 2016, **36**, 1210.
49. M. Shardell, D. Alley, G. Hick, S. El-Kamary, *Nutr. Res.*, 2011, **31**, 178.
50. K. M. Ranard, S. Jeon, E.S. Mohn, J.C. Griffiths, E.J. Johnson, J.W. Erdman Jr, *Eur. J. Nutr.*, 2017, **56** (Suppl 3), 37.
51. J.R. Lupton, S.A. Atkinson, N. Chang, C.G. Fraga, J. Levy, M. Messina, D.P. Richardson, B. van Ommen, Y. Yang, J.C. Griffiths, J. Hathcock, *Eur. J. Nutr.*, 2014, **53** (Suppl.1), 1.
52. A. Shao, J.N. Hathcock, *Regul. Toxicol. Pharmacol.*, 2006, **45**, 289.

Table 12.1 Carotenoid intake (μg per person per day) from national surveys.

	Spain ^{44,45}	Brazil ⁴⁶	U.S.A ⁴⁴
<i>Lutein + zeaxanthin</i>			
Total diet	1235		1356
From vegetables	776.4	704.1 ^a	
From fruits	63.8	128.8 ^b	
<i>Lycopene</i>			
Total diet	3056		5263
From vegetables	2637.6	361.1	
From fruits	329.7	295.6	
<i>α-carotene</i>			
Total diet	269		379
From vegetables	255.2	115.6	
From fruits	13	47	
<i>β-carotene</i>			
Total diet	1459		1942
From vegetables	1224.1	776	
From fruits	80.9	141.5	
<i>β-cryptoxanthin</i>			
Total diet	322		82
From vegetables	15.7	0.1	
From fruits	200.5	126.1	

^a 701 μg lutein person⁻¹ day⁻¹ and 3.1 μg zeaxanthin person⁻¹ day⁻¹.

^b 75 μg lutein person⁻¹ day⁻¹ and 53.8 μg zeaxanthin person⁻¹ day⁻¹.

Table 12.2 Approximate intake of free and ester forms of xanthophylls by the Spanish and Brazilian population.

Foods	µg xanthophylls per g of food		Food intake (g)		Spain (µg)		Brazil (µg)	
	free	esters	Spain	Brazil	free	esters	free	esters
Apple	0.4	2.1	41.4		17.8	86.7		
Mango	30.4	33.3		4.7			142.9	156.5
Orange	33	81	34.6	20.6	1141.8	2802.1	679.8	1668.6
Papaya	87.9	261.4	0.9	6.4	79.1	235.3	562.6	1673.0
Peach	1.5	1.6	15.3		23.0	24.5		
Pepper (red)	122.3	171.9	14.4		1761.1	2475.4		
Persimmon	0.9	12.4	0.6		0.5	7.4		
Tangerine	2.6	8.5	9.8	37.9	25.5	83.3	142.9	322.2

Data were calculated from refs. 44-46 and data from chapter 7 of this book.