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Review article

Nutritional and clinical relevance of lutein in human health

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Lutein is one of the most widely found carotenoids distributed in fruits and vegetables frequently consumed. Its presence in human tissues is entirely of dietary origin. Distribution of lutein among tissues is similar to other carotenoids but, along with zeaxanthin, they are found selectively at the centre of the retina, being usually referred to as macular pigments. Lutein has no provitamin A activity in man but it displays biological activities that have attracted great attention in relation to human health. Epidemiological studies have shown inconsistent associations between high intake or serum levels of lutein and lower risk for developing cardiovascular disease, several types of cancer, cataracts and age-related maculopathy. Also, lutein supplementation has provided both null and positive results on different biomarkers of oxidative stress although it is effective in increasing macular pigment concentration and in improving visual function in some, but not all, subjects with different eye pathologies. Overall, data suggest that whereas serum levels of lutein have, at present, no predictive, diagnostic or prognostic value in clinical practice, its determination may be very helpful in assessing compliance and efficacy of intervention as well as potential toxicity. In addition, available evidence suggests that a serum lutein concentration between 0·6 and 1·05 μmol/l seems to be a safe, dietary achievable and desirable target potentially associated with beneficial impact on visual function and, possibly, on the development of other chronic diseases. The use of lutein as a biomarker of exposure in clinical practice may provide some rationale for assessing its relationship with human health as well as its potential use within the context of evidence-based medicine.

Lutein: Biomarkers: Macular pigments: Evidence-based medicine

Lutein is a plant pigment that belongs to the well-known group of carotenoids. Man is not capable of synthesizing carotenoids *de novo* and, thus, their presence in human tissues is entirely of dietary origin, although man is capable of modifying some of them to some extent. Lutein is, along with β-carotene, one of the most widely distributed carotenoids in fruits and vegetables frequently consumed by different populations (Granado *et al.* 1996; O'Neill *et al.* 2001). Chemically, lutein and its structural isomer zeaxanthin are the dihydroxy derivatives of α-carotene and β-carotene, respectively, presenting two hydroxyl groups at the terminal rings of the molecule (Fig. 1), and thus are referred to as xanthophylls. The presence of substituted terminal β-rings in the molecule, however, also confers a higher polarity, which determines, in part, distinctive characteristics during absorption, transport, metabolism and deposition in tissues (Erdman *et al.* 1993; Castenmiller & West, 1998; Parker *et al.* 1999).

In foods, lutein can be found either in its free form, bound to proteins, or esterified as a monoester or di-ester (Klaui & Bauerfeind, 1981; Goodwin & Britton, 1988). After being released from the food matrix, it is incorporated into micelles to be absorbed by passive transport by enterocytes and, along with other carotenoids and other fat-soluble dietary components, is incorporated into nascent chylomicrons for transport to the liver. In blood, lutein is transported by lipoproteins with an even distribution among the different classes (i.e. LDL and HDL) (Parker, 1996). In addition, because of its polarity, it is assumed to be located at the lipoprotein surface and, thus, is more readily transferred among the different classes of lipoproteins even during postprandial metabolism (Parker *et al.* 1999).

Although distribution of lutein among tissues is similar to that of other carotenoids and is determined at least partly by LDL-receptor density, both lutein and zeaxanthin

Abbreviations: ARMD, age-related macular degeneration; CVD, cardiovascular disease; FCT, food composition table; MPOD, macular pigment optical density.

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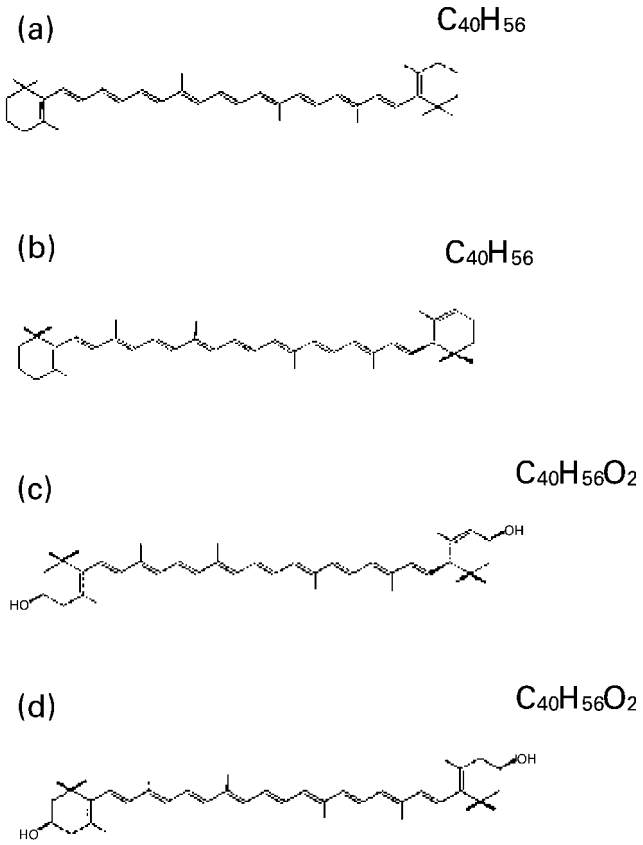

Fig. 1. Chemical structures of α-carotene (a), β-carotene (b), lutein (c) and zeaxanthin (d).

are selectively accumulated in different parts of the human eye (Bone *et al.* 1985; Handelman *et al.* 1988; Yeum *et al.* 1995; Bates *et al.* 1996; Rapp *et al.* 2000). Lutein and zeaxanthin constitute by far the major carotenoids present in these tissues where, in addition, highly specific binding proteins for these two carotenoids have recently been detected (Yemelyanov *et al.* 2001). Lutein and zeaxanthin are especially abundant at the centre of the retina (macula) and both xanthophylls are usually referred to as macular pigments.

Nutritional relevance: is lutein biologically active in man?

For a long time, nutrients have been widely considered in terms of their essentiality to man, in the interests of avoiding nutritional deficiencies. Traditionally, the nutritional importance of carotenoids in man has rested on their provitamin A activity (the capacity to be converted into vitamin A) exhibited by some of them (i.e. β-carotene, α-carotene and β-cryptoxanthin). However, because of the presence of hydroxylated terminal rings in its structure, lutein does not fit the structural requirements for this activity and, thus, shows no such activity in man (Zechmeister, 1962; Simpson, 1983), a fact that probably constitutes the major reason its potential relevance in human health had long been overlooked.

Nevertheless, since the 1950s, the quest for optimal health, not the mere avoidance of deficiency, combined with the development of analytical techniques and nutritional epidemiology, which showed the potential effects of many food components as preventive or risk factors in relation to chronic and degenerative diseases, changed this context. Nowadays, nutritional interest in lutein is based not on its 'essentiality' but on: (1) the biological activities it shows, which may be potentially relevant to human health; (2) its presence in foods frequently consumed and, thus, the possibility of manipulating its intake by several dietary approaches, with a potentially important impact on human health, disease prevention and cost savings. For example, concerning age-related eye diseases where lutein may play a beneficial role, pooled data from North America, Europe and Australia provide estimates of about 0·2 % of individuals aged 55 to 64 years and 13 % of those aged 85 years or older to have age-related macular degeneration (ARMD; Flood *et al.* 2002). Since the elderly is an increasing proportion of the population, it is estimated that in the USA about 6·3 million individuals will have ARMD by the year 2030 (Seddon & Hennekens, 1994). So, identifying preventive factors for ARMD is especially important because treatment possibilities are limited both in scope and effectiveness. Similarly, cataract is one of the major causes of preventable blindness throughout the world with an increase in prevalence (in the USA) from approximately 5 % at age 65 years to about 50 % for individuals older than 75 years. In this sense, it is estimated that a delay in cataract formation of about 10 years would reduce the prevalence of visually disabling cataract by about 45 %, enhancing the quality of life for much of the world's older population and substantially reducing the economic burden (US$ 5–6 billion) due to cataract-related disability and cataract surgery (Taylor & Hobbs, 2001).

According to the criteria of Bendich & Olson (1989), to establish the potential role of lutein in human health, its biological activities can be characterized as functions, actions and associations. Functions relate to the essential role the nutrient plays at physiological concentrations. In terms of nutrition, functions refer to the essentiality of a nutrient and thus its capacity to prevent deficiency states. Since the only proven function of carotenoids in man is the provitamin A activity of some of them, lutein is not considered an essential nutrient for man. In fact, to our knowledge, there is no clinical condition reported in man specifically associated with lutein deficiency or toxicity, apart from the (reversible) hypercarotenaemia, with or without carotenodermia (skin pigmentation), usually due to an excessive intake, although it may also be associated with some pathological conditions (i.e. diabetes mellitus, anorexia nervosa) (Rojas-Hidalgo, 1987).

Actions usually refer to effects (beneficial or adverse) displayed by a given component when tested under non-physiological conditions (usually using 'pharmacological' doses) *in vitro*, *ex vivo*, in cell cultures or in animal models. As observed with other carotenoids, a number of biological actions have been reported for lutein under different assay conditions including cell–cell communication, inhibition of cell transformation, inhibition of the

monocyte-mediated inflammatory response, immune enhancement, *in vitro* antioxidant activity, inhibition of LDL resistance to oxidation and macula protection (Bendich & Olson, 1989; Sies *et al.* 1992; Chopra & Thurnham, 1993; Bertram, 1994; King *et al.* 1997; Park *et al.* 1998; Beatty *et al.* 1999; Van den Berg *et al.* 2000; Collins, 2001; Dwyer *et al.* 2001).

Many aspects of the metabolism of carotenoids in man remain largely unknown. However, dehydration products (anhydrolutein), geometric isomers and ester forms of lutein have been reported in human serum and tissues, as have several putative oxidative metabolites, referred to as ketocarotenoids (Khachik *et al.* 1992, 1997*a*,*b*). Since these ketocarotenoids are not widely found in foods frequently consumed and their concentrations in serum increase upon lutein supplementation, it is suggested that these carotenoids are formed *in vivo* (Khachik *et al.* 1995; Olmedilla *et al.* 1997*a*). In this respect, it has been proposed that lutein and zeaxanthin (macular pigments) may prevent light-initiated oxidative damage to the retina and retinal pigment epithelium and thus protect against age-related deterioration (Hammond *et al.* 1998; Beatty *et al.* 1999). However, it must be pointed out that while these actions provide biological mechanisms by which lutein may exert its action, extrapolation to *in vivo* situations is difficult and these actions must be demonstrated in human subjects and their relevance established under *in vivo* physiological conditions.

The term associations refers to epidemiological evidence of a correlation between nutrient exposure (lutein) and health or disease outcomes. Epidemiological associations have been reported between lutein and several highly prevalent diseases in developed countries, namely cardiovascular diseases (CVD), several types of cancer and age-related cataracts and macular degeneration (see later, p. 492). These associations, however, do not provide a 'causal' link but basically statistical or epidemiological relationships.

Summarising, regardless of the lack of provitamin A activity, the biological actions displayed by lutein, along with the epidemiological evidence in relation to chronic and degenerative diseases, has triggered the interest in this carotenoid and its consideration as a potentially beneficial phytochemical with relevance to human health. In addition, the differential characteristics of lutein (and zeaxanthin), namely the selective accumulation in the human eye and the presence of binding proteins with high affinity for these xanthophylls, support the biological plausibility of a relevant role in human health.

Assessment of lutein exposure

Epidemiological associations are based on the relationships between nutrient exposure and intermediate endpoints during the course of disease or clinical outcomes. To establish a causal relationship between a nutrient and the clinical endpoint, it is assumed that an increased exposure to that nutrient, usually measured by intake or serum levels, is capable of enhancing a relevant biochemical indicator and/or function (enhanced function, reversible), which, in turn, is considered to be causally related to the modification of a disease process (structural alterations, reduced disease risk) that is causally related to the health outcome (Van't Veer & Kok, 2000).

Traditionally, carotenoid (namely lutein) exposure or nutritional status assessment in human subjects has been routinely performed by dietary or biochemical methods, both of which have advantages and limitations. More recently, for research purposes, non-invasive psychophysical tests using heteroflicker photometry (Hammond *et al.* 1997; Landrum *et al.* 1997; Beatty *et al.* 2001) and Raman spectroscopy (Bernstein *et al.* 1998, 2002) is being used to measure macular pigment optical density (MPOD), which provides information on long-term lutein (and zeaxanthin) exposure. However, while these new tools may be highly valuable, at present, the instruments are not commercially available for routine nutritional or ophthalmological assessment. In addition, because of the selective deposition of these carotenoids in this tissue (retina), its relevance to other tissues, intermediate biomarkers and (non-ocular) clinical conditions remains to be established.

Dietary intake assessment

Although about forty to fifty carotenoids are available in the diet to be absorbed, metabolized or utilized by man, only five or six are routinely measured in foods and human serum and tissues. Foods traditionally considered as good sources of lutein are green vegetables, especially dark green varieties such as spinach, broccoli, beet, lettuce, etc. (Heinonen *et al.* 1989; Granado *et al.* 1992; Chug-Ahuja *et al.* 1993; Hart & Scott, 1995). As occurs with other carotenoids, dietary intake of lutein shows a high variability within and between subjects and among populations (Thurnham, 1988; Ito *et al.* 1990; Hercberg *et al.* 1994; Granado *et al.* 1996; Agudo *et al.* 1999; National Academy of Sciences Institute of Medicine, 2000; O'Neill *et al.* 2001; Johnson-Down *et al.* 2002), which relates to both natural or intrinsic factors (dietary changes, seasonality) and methodological aspects (Granado *et al.* 1997; Rodriguez-Amaya, 1997; Deharveng *et al.* 1999).

Regardless of the confidence in the method used for dietary assessment, evaluation of nutrient exposure by dietary means is ultimately based on the availability of reliable data on food composition. Since the nutritional interest in carotenoids was largely due to their pro-vitamin A activity, traditionally, food composition tables (FCT) and databases did not include values for individual carotenoids in foods, although they were considered for vitamin A (retinol equivalents) content. However, the increasing evidence of the potential role of several constituents of fruits and vegetables (carotenoids) in human health led to a revision of former data and the inclusion of non-provitamin A carotenoids (i.e. lutein) in the new FCT and databases during the 1990s (Table 1).

However, in addition to the variability observed in lutein intake, several methodological factors compromise the homogeneity and comparability of the carotenoid content in foods, even in the most recently developed databases and despite the use of highly specific (HPLC) methods. Some of the factors affecting the reliability of the available

Table 1. Availability of data for lutein and lutein + zeaxanthin content in foods: nutritional and epidemiological studies

Reference	Type of report	Country (food origin)	Lutein	Lutein + zeaxanthin
Khachik *et al.* (1989)	HPLC report	USA	Y	
Heinonen *et al.* (1989)	HPLC report	Finland		Y
Tee & Lim (1991)	HPLC report	Malaysia	Y	
Granado *et al.* (1992)	HPLC report	Spain	Y	
Poorvliet & West (1993)	Database	Several		Y
Mangels *et al.* (1993)	Database	USA (several)		Y
Hart & Scott (1995)	HPLC report	UK	Y	
Olmedilla *et al.* (1996)	Database	Spain	Y	
Sommerburg *et al.* (1998)	HPLC report	USA	Y	
Holden *et al.* (1999)*	Database	USA (USA)		Y
Murkovic *et al.* (2000)	Database	Austria		Y
O'Neill *et al.* (2001)	Database	Europe (several)		Y

* Zeaxanthin values reported independently for selected foods.

data have been pointed out elsewhere (Granado *et al.* 1997; Rodriguez-Amaya, 1997; Deharveng *et al.* 1999), and are analytical or food-related.

Analytical factors affecting the reliability of the data are that:

most reports and databases provided combined data for lutein plus zeaxanthin, leading to an overestimation of the 'true' content of lutein in several foods, so that while the presence of zeaxanthin may be negligible in most green vegetables, this is not so in many fruits frequently consumed where equal or higher amounts of zeaxanthin are present (i.e. oranges);

in most if not all FCT, it is not indicated whether the value for lutein refers to free or total (saponified) content. Since lutein is also present in ester forms, especially in fruits, this constitutes a major source of uncertainty that may lead to a substantial underestimation of the 'true' lutein content;

despite it being more accurate, precise and expensive than other techniques, the use of HPLC does not ensure a good performance of the analysis or increase the confidence of the data. Results from different international inter-laboratory exercises for carotenoid analysis in foods showed a wide variability in the data (CV of 18-55 % for lutein), even among expert laboratories and despite the use of common standards and 'common' extraction protocols (Van den Berg *et al.* 2000).

Food-related factors basically concern:

the identity of the food (i.e. different origin, different varieties);

the part of the plant consumed (i.e. inner or outer leaves);

ripeness, which dramatically affects xanthophyll synthesis (content), especially in some fruits;

food processing, which affects retention and degradation, isomerization and bioavailability (Granado *et al.* 1997; Rodriguez-Amaya, 1997).

When evaluating the amount of a nutrient consumed (nutrient exposure), it is important to note that it depends not just on the content in food but on the amount of the food consumed and, most importantly, the frequency of consumption. This is essential to identify the major contributors of nutrient (lutein) intake. In this regard, Table 2 shows our own estimations using data obtained from a European multicentre study, where dietary intake was estimated using a common food-frequency questionnaire and database of carotenoids in food (O'Neill *et al.* 2001). As shown, although green vegetables are important contributors to lutein intake in five European

Table 2. Ten top contributors (%) to lutein (+zeaxanthin) intake in five European countries (adapted from AIR Study Final Report (1997) and O'Neill *et al.* 2001)*

Spain (*n* 70)	France (*n* 76)	UK (*n* 71)	Republic of Ireland (*n* 76)	The Netherlands (*n* 75)
Spinach (34)	Spinach (31)	Peas (36)	Peas (19)	Spinach (30)
Lettuce (16)	Lettuce (8)	Broccoli (8)	Broccoli (16)	Broccoli (10)
Oranges (7)	Eggs (8)	Eggs (8)	Eggs (10)	Peas (9)
Eggs (7)	Mix veg (6)	Sweetcorn (7)	Carrots (9)	Chicory (8)
Broccoli (6)	Cucumber (6)	Lettuce (6)	Tomato (8)	Lettuce (4)
Peas (6)	Green beans (4)	Carrots (4)	Oranges (7)	Tomato (4)
Potatoes (3)	Courgette (4)	Tomato (4)	Peppers (6)	Oranges (4)
Tangerines (3)	Peas (3)	Tangerines (4)	Sweetcorn (4)	Eggs (4)
Peppers (3)	Tomato (3)	Celery (4)	Spinach (3)	Green beans (4)
Leeks (2)	Sweetcorn (2)	Spinach (3)	Lettuce (3)	Potatoes (4)
Total (97)	Total (75)	Total (84)	Total (85)	Total (81)
Green veg (67)	Green veg (56)	Green veg (57)	Green veg (47)	Green veg (65)

veg, vegetables.
* Assessed in winter.

groups, relative contribution differs substantially among them. It is also worth noting the relative contribution of non-green vegetables and fruits and the fact that non-green foods may account for almost half of the total lutein intake in some groups. More importantly, zeaxanthin, a xanthophyll probably as important as lutein in relation to visual function, is obtained almost exclusively from non-green vegetables in certain population groups (i.e. Spaniards; Table 3), as previously reported (Granado *et al.* 1996).

Major contributors to lutein intake differ widely both within and between populations, and their misidentification may substantially affect the variability of lutein intake. Moreover, their inclusion in or exclusion from the questionnaires may lead to the over- or underestimation of the 'true' intake, providing misleading results in the evaluation of nutrient exposure and contributing to the misclassification of subjects, to uncertainty and to inconsistencies between observational studies based on dietary methods.

Biochemical markers

The use of biochemical indicators overcome most of the confounding factors and biases associated with dietary methods. They are considered more reliable since they provide more accurate information on the amount of nutrient available to tissues in which these nutrients may exert their biological action. Regarding concentrations of lutein in human tissues, data are scarce and mostly refer to tissues that can be sampled non-invasively (buccal mucosa cells, human milk or adipose tissue). With the exception of human retina, where concentrations of lutein and zeaxanthin may be measured by non-invasive techniques (see later; p. 489), the available data on tissues subject to involvement in diseases that have been associated with lutein in epidemiological studies (i.e. cancer, CVD) are very scarce and usually refer to reports on very small numbers of subjects, sometimes at autopsy, or biopsies of different tissues (normal or tumour) examined under different analytical conditions, circumstances that highly compromise their comparability (Kaplan *et al.* 1990; Nieremberg & Nann 1992; Stahl *et al.* 1992, 1993; Schmitz *et al.* 1993). Moreover, because sample collection is invasive and its relevance to the disease uncertain, the determination of the carotenoid profile in tissues is not performed or substantiated for clinical testing except, possibly, in the case of using psychophysical methods to determine the MPOD for research purposes.

Table 3. Top contributors (%) from fruit and vegetables to lutein and zeaxanthin intake in Spain (from Granado *et al.* 1996)*

Lutein	Zeaxanthin
Spinach (27)	Orange (58)
Beet (22)	Potato (27)
Orange (12)	Spinach (12)
Potato (12)	
Lettuce (11)	
Green bean (6)	
Artichoke (4)	
Tomato (2)	
Total (96)	Total (97)
Green vegetables (70)	Green vegetables (12)

* Assessed in winter.

In the absence of accepted, specific and validated functional methods for the assessment of lutein status, the serum concentration is widely used as the 'best available' method to establish the nutritional status of lutein in human subjects on a large scale. However, despite being the biochemical marker most widely used, it also presents several limitations. The serum lutein concentration relates to dietary intake and thus shows a wide variability both within and between subjects and among populations, reflecting the variation in food (nutrient) intake or subject response. As is the case with other carotenoids, it is considered to reflect short-term dietary intake, although it is widely accepted as a good biomarker of fruit and vegetable intake (Ascherio *et al.* 1992; Hercberg *et al.* 1994; Olmedilla *et al.* 1994; Thurnham *et al.* 1998). Lutein in serum correlates with lutein intake although the degree of this association (diet–serum) varies widely depending on a number of factors (Olmedilla *et al.* 1994; Scott *et al.* 1996). However, although a correlation between intake, serum and some tissues exists (Yong *et al.* 1994; Johnson *et al.* 2000; Curran-Celentano *et al.* 2001; Broekmans *et al.* 2002), intake assessment and serum concentrations do not necessarily reflect the amounts in tissues or the relative contribution of carotenoids in tissues, especially regarding isomer distribution (structural and geometrical isomers) (Stahl *et al.* 1993; Su *et al.* 1998).

Specifically, in serum, lutein:zeaxanthin is about 3:1, whereas in macula, this ratio reaches values of up to 1:2 and shows a specific concentration pattern from the centre to the eccentric region (Bone *et al.* 1997; Landrum *et al.* 1999). In addition, meso-zeaxanthin, the major form of zeaxanthin in retina, is probably the result of chemical processes occurring within the eye, possibly a conversion product derived from lutein at the tissue level (Bone *et al.* 1997).

A high analytical variability also exists in serum carotenoid analysis (Van den Berg *et al.* 1993), in addition to the simultaneous quantification of lutein and zeaxanthin. Most analytical methods, including many used in epidemiological studies, do not separate lutein and zeaxanthin from those referred to as ketocarotenoids (suggested oxidation products). Thus, if the latter are considered potential metabolites of the *in vivo* antioxidant activity of lutein, many analytical methods report lutein+zeaxanthin+oxidation products as a single compound (lutein), leading to the overestimation of the 'true' concentration (nutrient exposure) and forfeiting the ability to detect potential markers of the *in vivo* biological (antioxidant) activity of lutein. Despite the fact that these 'metabolites' have been reported to be present in serum and tissues (retina) (Khachik *et al.* 1997*b*; Bernstein *et al.* 2001) and despite their increment upon lutein supplementation (Khackik *et al.* 1995; Olmedilla *et al.* 1997*a*), to date they have not been used as potential biomarkers of lutein (antioxidant) activity or assessed in relation to any biomarker and/or disease process. Therefore, their physiological relevance, if any, remains to be elucidated.

Evidence of the relationship between lutein and human health

Epidemiological evidence supporting a potential role of lutein in preventing chronic and degenerative diseases comes from different types of studies, mostly observational, while intervention trials are experimental and on a (very) small scale. Few observational studies have examined associations between exposure to lutein (individually), assessed by dietary intake or serum levels, and diseases with high prevalence in developed countries, and most are of case-control and cross-sectional design. On interpreting the available evidence, several points should be kept in mind: (1) despite the huge number of studies dealing with the protective effect of fruit and vegetable consumption, few have dealt specifically with lutein and, in these, the clinical endpoints differ substantially; (2) most of these studies have been carried out within the last 10 years due to the availability of accurate information about lutein content in food databases and the use of HPLC analysis on a large scale; (3) interpretation of these studies should be done with some caution because of the uncertainties associated with the assessment of nutrient exposure, as mentioned earlier (p. 489).

Studies dealing with cancer are scarce and refer to different types and locations. Results show an inverse association between high lutein intake and/or higher serum levels and risk for lung cancer (Michaud *et al.* 2000; Ratnasinghe *et al.* 2000; Voorrips *et al.* 2000) whereas no association or inconsistent results are reported for dietary or serum levels and cancer at other locations (prostate, breast, colon, bladder and stomach) (Giovannucci *et al.* 1995; Dorgan *et al.* 1998; Yeum *et al.* 1998; García *et al.* 1999; Botterweck *et al.* 2000; Slattery *et al.* 2000; Toniolo *et al.* 2001; Zeegers *et al.* 2001). For CVD, results regarding early atherosclerosis and CHD are controversial, with null (Kohlmeier *et al.* 1997; D'Odorico *et al.* 2000; Klipstein-Grobush *et al.* 2000) and protective associations (Street *et al.* 1994; Howard *et al.* 1996; Iribarren *et al.* 1997; Dwyer *et al.* 2001), whereas for stroke the data suggest a protective role (Ascherio *et al.* 1999; Hirvonen *et al.* 2000); the number of studies, however, is small and those available refer to case-control analyses where nutrient exposure may be altered due to the disease process. In relation to age-related eye diseases, the results suggest both a protective and no association whatsoever between lutein intake or serum levels and the development or prevalence of cataracts (Mares-Perlman *et al.* 1995*a*; Brown *et al.* 1999; Chasan-Traber *et al.* 1999; Lyle *et al.* 1999; Gale *et al.* 2001; Taylor *et al.* 2002), and those from studies based on serum levels are highly inconsistent. Similarly, inconsistent associations have been reported between dietary intake and serum levels, and the presence of ARMD (Eye Disease Case-Control Study Group 1993; Seddon *et al.* 1994; Mares-Perlman *et al.* 1995*b*; Beatty *et al.* 2001; Flood *et al.* 2002).

In general, results from observational studies are somewhat scarce and rather inconsistent, a fact that may be related to a number of factors including the use of different clinical endpoints, populations assessed and study designs (inclusion criteria, duration, amounts ingested), inaccuracies in nutrient exposure assessment (diet and serum levels), lack of biomarkers of nutritional status with relevance in tissues, a 'temporal' gap as confounding factor (serum levels reflect short-term exposure), the presence of the disease affecting the biomarker and the fact that the potential effect of a nutrient is specific for a certain disease, stage of a disease and/or 'target' or susceptible groups.

Supplementation studies in human subjects

Although not considered the 'gold standard', intervention trials add strong support to a nutrient–health relationship, make it possible to establish temporality and specificity in the causal pathway and allow interpretation of results in terms of cause and effect. However, although concentrations of lutein and zeaxanthin in serum and tissues (i.e. macula) have been shown to increase significantly upon ingestion of lutein-rich foods and/or lutein capsules (Hammond *et al.* 1997; Landrum *et al.* 1997; Olmedilla *et al.* 1997*a*; Granado *et al.* 1998; Johnson *et al.* 2000), lutein has not been used on a large scale in intervention trials to test its efficacy in relation to chronic diseases.

Based on the hypothesis that oxidative stress is involved in tissue damage and the development of chronic diseases, in the mid-1990s a European multicentre, placebo-controlled supplementation study was conducted to test whether dietary antioxidants (i.e. carotenoids) could provide protection against oxidative stress and thus reduce the risk of oxidative tissue damage. In this study, to our knowledge the largest and widest performed to date, a total of 400 non-smokers (200 men, 200 women, aged 25–45 years) from five European countries, with different diets and lifestyles, were assigned to supplement their diets for 4 months with one of three carotenoids (lutein, α- + β-carotene or lycopene) or placebo (100 subjects/group). Because of the long-term progression of chronic and degenerative diseases, several biomarkers of oxidative damage associated with CVD and cancer were assessed during the study, including DNA, lipids and protein oxidative damage, as well as enzymic and soluble antioxidants in plasma and serum. In this study, lutein supplementation was followed, on average, by a five- to six-fold increase in serum lutein, with maximum values observed 3 to 4 weeks later, after which a steady-state (plateau) level was reached (Olmedilla *et al.* 2002). Ketocarotenoid and zeaxanthin concentrations in serum also increased and, interestingly, above certain serum lutein concentrations (1·05 μmol/l; 600 μg/l), ester forms of lutein appeared in serum (Granado *et al.* 1998). However, the effect of lutein supplementation provoked no changes in several biomarkers of oxidative damage to DNA, lipids and proteins or in other soluble and enzymic antioxidants including ascorbic acid, α-tocopherol, glutathione, superoxide dismutase or glutathione peroxidase (Collins *et al.* 1998*a*,*b*; Hininger *et al.* 2001). Thus, on the basis of these data, it seems that, in those apparently healthy subjects, lutein supplementation increases serum levels of certain carotenoids, but this effect is not associated with any change in different biomarkers of oxidative stress (Olmedilla *et al.* 2002).

Another study, however, using other intermediate biomarkers does not confirm this apparent lack of effect. Recently, on the basis of epidemiological evidence of an inverse association between serum lutein levels and progression of the intima-media thickness of carotid arteries (in human subjects) it was shown that lutein inhibited the inflammatory response of monocytes to LDL trapped in the artery wall and reduced monocyte migration (co-culture), while indicators of oxidative stress (lipid hydroperoxides and erythrocyte fragility) were reduced and LDL was markedly resistant to oxidation by lutein supplements (in mice) (Dwyer *et al.* 2001). So, while these findings are very encouraging, suggesting a potential role of lutein in the prevention of the development of early atherosclerosis, they also highlight the inconsistency of results from studies with different designs and raise important concerns about the usefulness, methodology, relevance and validity of the different biomarkers of oxidative stress in relation to the disease process. In this regard, since no one animal model completely mimics human absorption and metabolism of carotenoids, and for studies using biomarkers of heart disease primates and gerbils are probably more appropriate than rats and mice (Lee *et al.* 1999), extrapolation of these results and their relevance to man should be considered with caution.

Lutein has been used in the treatment of eye diseases and to protect visual function since the 1950s (Nussbaum *et al.* 1981). Small supplementation studies have been performed, mostly in subjects with compromised visual function, and the results appear to be promising, although the studies differ in terms of design, endpoints measured and subjects involved (Table 4). In control subjects and patients with minimally compromised visual function, lutein supplementation provokes an increase in lutein concentrations in serum and in macula, as measured by MPOD, although it is not observed in all subjects. Similarly, effects on visual function are also variable with improvements and no effect being reported. These individuals, however, may clear the lutein from serum very quickly, making the rise and fall difficult to detect in serum and, in addition, the retina may not be the main body pool or the levels are tightly regulated in this tissue. In subjects with visual impairment, promising results have been obtained but, again, the studies involved few subjects and differing protocols. Many of these studies refer to subjects suffering from retinitis pigmentosa, an inherited disease leading to retinal degeneration and blindness, in whom lutein supplementation led to a better performance of visual function, but not in all subjects (Aleman *et al.* 2001). In a few patients with age-related cataracts, lutein supplementation, but not α-tocopherol, improved visual acuity (Olmedilla *et al.* 2003), whereas not all ARMD patients showed improvement in visual function, possibly because their visual function was highly compromised (Olmedilla *et al.* 2001*a*; Flood *et al.* 2002).

It is important to note that, in several studies involving different supplementation protocols (Hammond *et al.* 1997; Landrum *et al.* 1997; Johnson *et al.* 2000; Olmedilla *et al.* 2001*a*), serum lutein concentrations reached and time required to observe a response (lutein in serum and MPOD) were consistent and that there were parallel improvements in clinical indicators relevant to disease (visual acuity in patients with cataracts), providing some valuable information on the dose, timing and methods to assess treatment efficacy.

Relevance of lutein in clinical practice and community-based interventions

The nutritional relevance of lutein in human health may be summarized as follows: it is a non-provitamin A carotenoid but a biologically active phytochemical in man. Consequently, interest in lutein is increasing. Since there are no human pathological conditions associated with deficiency or toxicity specifically related to lutein and because lutein metabolism in man is still largely unknown, lutein status is not assessed in clinical laboratories these days. Furthermore, recommended or maximum tolerable intakes for lutein in human consumers have not been established (National Academy of Sciences Institute of Medicine 2000), despite the increasing commercial availability and use of lutein-containing supplements and the recommended intake by some physicians.

Lutein displays different biological actions *in vitro* and *ex vivo* (Chopra & Thurnham, 1993; Bertram 1994; King *et al.* 1997; Collins 2001). However, *in vivo* actions need to be proven in human subjects under physiological conditions. The consistency and strength of the associations may be considered relatively high and strong for age-related eye diseases but rather low and weak for CVD and cancer. To date, other causality criteria such as temporality and specificity are scarce and based on small supplementation trials for eye diseases.

Significance and usefulness of serum lutein concentrations

Interpretation of concentrations of lutein in serum is limited because of the variability and the lack of widely accepted reference ranges in (control) populations. In addition, lutein levels in serum are not (routinely) determined in clinical settings, constituting an important gap regarding the distribution of lutein in serum under pathological conditions. In this context, Fig. 2 shows the distribution of lutein in serum of 1800 patients clinically diagnosed with different conditions analysed in our laboratory. The results are grouped and broadly classified based on the major clinical condition (diagnosis) as received in the laboratory. Two observations, however, must be made regarding this classification: first, the samples are not necessarily representative of a wide range of clinical conditions since they were received only for (fat-soluble) vitamin analysis; second, it is probable that in many subjects other clinical or pathological conditions coexist with the major diagnosis reported for vitamin analysis.

Fig. 2, while not intended to be exhaustive and detailed, shows that the concentrations of lutein in serum display a non-parametric (biased to the lower end) wide distribution regardless of the condition (control or pathological) of the group. In relative terms, two groups may be considered in Fig 2: (1) patients with malabsorption syndromes, surgical

Table 4. Supplementation studies with lutein and visual function

Characteristics and state of subjects	*n*	Dose and time	Biomarker of modified function or modified disease process	Effect	Reference
Control subjects					
Normal dark adaptation, visual acuity	14	Lutein dipalmitate (Helenien, adaptinol) 2–6 months	Dark adaptation, visual acuity	Transiently improved	Monje *et al.* (1948)*
Normal patients		Helenien	Dark adaptation	Improved	Klaes & Riegel (1951)*
Normal patients		Helenien	Threshold sensitivity	Improved	Mosci *et al.* (1956)*
Normal patients		Helenien	Dark adaptation	Improved	Andreani *et al.* (1956)*
Controls		Helenien	Serum lutein levels	Increased	Hayano *et al.* (1959)*
			Dark adaptation	Improved	
Controls	11	60 g spinach ± 150 g maize for 4–15 weeks	Serum lutein and zeaxanthin levels	Increased	Hammond *et al.* (1997)
			MPOD	Increased (in 4 weeks)	
Controls	2	30 mg/d for 140 d	Serum lutein/zeaxanthin levels	Increased	Landrum *et al.* (1997)
			MPOD	Increased	
Controls	7	Spinach + maize for 15 weeks	Lutein in serum, BMC, adipose	Increased	Johnson *et al.* (2000)
			MPOD	Increased	
Controls	8	10 mg/d for 12 weeks	Serum lutein levels	Increased	Tos *et al.* (2000)
			MPOD	Increased	
Controls	8	20 mg lutein/d for 6 months	Serum lutein and zeaxanthin	Increased	Aleman *et al.* (2001)
			MPOD	Unchanged	
			Visual acuity	Unchanged	
			Foveal sensitivity	Unchanged	
Elderly controls	52	> 4 mg/d (regularly consumed)	MPOD (Raman spectroscopy)	Similar to younger adults	Bernstein *et al.* (2002)
Patients with visual impairment					
Retinitis pigmentosa		Helenien	Threshold sensitivity	Improved	Mosci *et al.* (1956)*
Retinitis pigmentosa		Helenien	Dark adaptation	Improved	Andreani *et al.* (1956)*
Retinitis pigmentosa		Helenien	Serum lutein levels	Increased	Hayano *et al.* (1959)*
			Dark adaptation	Improved	
Retinitis pigmentosa	18	Helenien	Electroretinograms	No change	Muller *et al.* (1961)*
Retinitis pigmentosa	21	20 mg lutein/d for 6 months	Serum lutein and zeaxanthin	Increased	Aleman *et al.* (2001)
			MPOD	Increased in 50 % of patients	
			Central vision (visual acuity, foveal sensitivity)	Unchanged	
Retinitis pigmentosa	13	20 mg lutein/d for 9 weeks	Visual acuity	Improved (not all subjects)	Dagnelie *et al.* (2000)
			Visual field area	Improved	
Progressive myopia, chorioretinopathy	50	Helenien	Luminous and chromatic sensitivity	Improved	Asciano *et al.* (1974)*
Cataract patients	5 (nine eyes)	15 mg × 3/week for 2 years	Serum lutein and zeaxanthin levels	Increased	Olmedilla *et al.* (2001*a*)
			Lutein esters in serum	Undetected	
			'Oxidative' lutein products	Increased	
			Visual acuity	Improved	
			Contrast sensitivity	Improved	
			Glare sensitivity	Improved	
ARMD patients	6	Spinach, lutein	Visual acuity	Improved	Richer (1999)
			Contrast sensitivity	Improved	
			Glare recovery	Improved	
ARMD patients	5 (nine eyes)	15 mg × 3/week for 1 year	Serum lutein and zeaxanthin	Increased	Olmedilla *et al.* (2001*a*)
			'Oxidative' lutein metabolites	Increased	
			Lutein esters in serum	Undetected	
			Visual acuity	No effect or slight improvement	
ARMD patients	15	> 4 mg/d (regularly consumed)	MPOD (Raman spectroscopy)	Improved (similar to non-ARMD patients)	Bernstein *et al.* (2002)

MPOD, macular pigment optical density; BMC, buccal mucosa cells; ARMD, age-related macular degeneration.
* Data taken from Nussbaum *et al.* (1981).

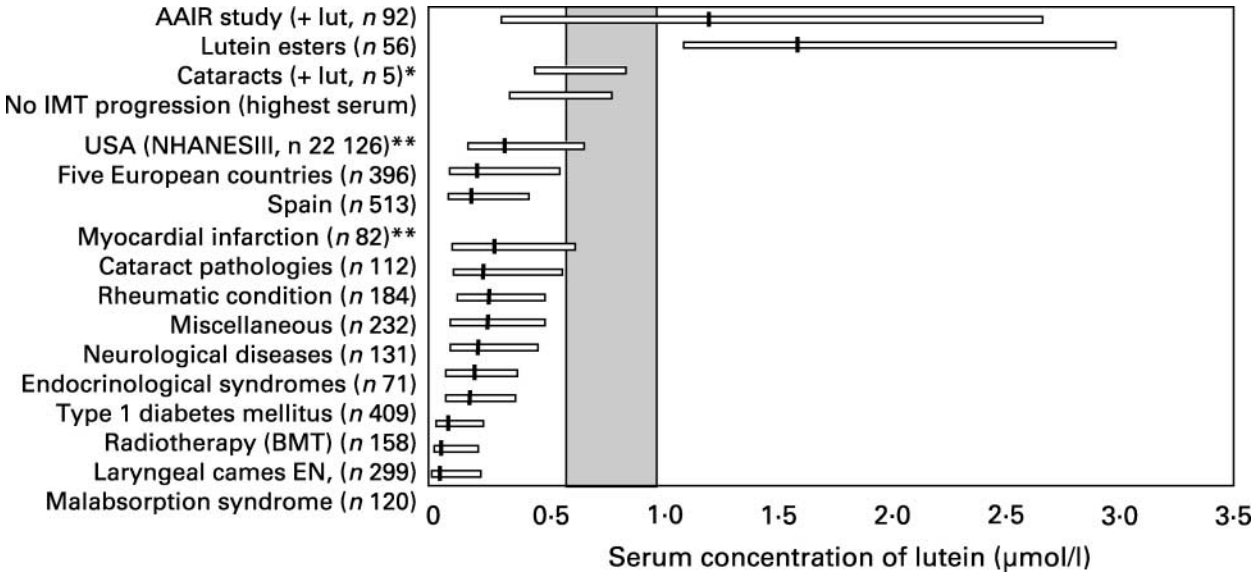

Fig. 2. Distribution (5–95th percentiles; □) of serum concentrations of lutein (lut) in control groups, subjects supplemented with lutein under different protocols and groups of patients clinically diagnosed with different conditions (see p. 493 and 495). (▮), Median values; (▮), safe level; AAIR, Agroindustrial Research Programme; *, range; IMT, intima-media thickness; **, lutein + zeaxanthin; BMT, bone marrow transplantation; EN, enteral nutrition. (Data taken from Olmedilla *et al.* 1997*b*, 2001*a*,*b*; Granado *et al.* 1998; National Academy of Sciences Institute of Medicine, 2000; Dwyer *et al.* 2001; F Granado, B Olmedilla and I Blanco, unpublished results.)

stress (laryngeal cancer, before and after enteral nutrition) or receiving radiotherapy (before bone marrow transplantation) show ranges and median concentrations in the lower part of reference values in control groups; (2) patients who have lutein levels (range and median) close to (type 1 diabetes mellitus, other endocrinological syndromes, neurological disorders) or slightly above those observed in control groups (rheumatic conditions, after acute myocardial infarction, senile cataracts and other clinical conditions).

Based on these data and in comparison with apparently healthy controls, the serum lutein concentrations observed in these groups are of uncertain clinical relevance. Lutein levels in serum should be carefully interpreted since they may be altered because of the disease process and thus do not provide reliable information about the implication, if any, in the onset and/or progression of the disease. Moreover, because of its transport by plasma lipoproteins, lutein levels in serum may be misleading as they may be secondary to other physiological and biochemical events associated with certain conditions such as the acute-phase response (i.e. myocardial infarction) or drug use (i.e. lipid-lowering drugs) or with certain populations (i.e. elderly individuals). From a clinical perspective, it seems clear that lutein concentrations in serum have little or no predictive, diagnostic or prognostic value in a variety of disease processes; this is also applicable when the potential role of lutein as a risk or preventive factor is examined in epidemiological (i.e. case-control) studies.

Nevertheless, despite these constraints, lutein concentrations in serum may be useful as a 'marker' both on the clinical and the community level. For example, lutein supplementation increases MPOD and is associated with a better functional (visual) performance in some, but not all, patients with a number of clinical conditions (cataracts, ARMD, retinitis pigmentosa) (Aleman *et al.* 2001; Olmedilla *et al.* 2001*a*), although this effect does not necessarily mean that the disease process has been modified as a result of the intervention (i.e. cataracts still develop) (Olmedilla *et al.* 2001*a*). While the functional effect (i.e. visual performance) is the primary objective in these interventions, the assessment of serum levels may be very useful in terms of checking compliance and thus efficacy of the intervention, as well as for monitoring and establishing relationships between changes in serum and those in functional and intermediate biomarkers relevant to the disease. Moreover, information on serum changes may also be important in order to establish and adjust effective doses, thresholds for treatment efficacy, timing of the intervention (required for a functional effect), risk evaluation (saturation or potential adverse effects) derived from long-term interventions and prevention of unforeseen additional risks particularly with regard to supplementation.

Measurement of lutein exposure is also relevant on a community basis. Nowadays, one of the major strategies in health promotion is focused on adequate strategies in preventive medicine, that is, to avoid risk factors and promote healthy behaviours. Increased lifespan and incidence of several chronic diseases run in parallel and, thus, prevention is the most convenient, sustainable and cost-effective strategy to delay the onset and progression of these conditions on a population basis.

Dietary assessment methods may be appropriate and useful for estimating lutein exposure on a group or community basis, although, because of the relevant constraints especially regarding FCT and the misuse of these data, this approach may not be suitable on an individual level. Serum lutein, when assessed repeatedly, prospectively on a long-term basis, may be a good indicator of healthy

(eating) habits related to chronic diseases. On a community level, screening of lutein concentrations in serum would allow the identification and establishment of appropriate cut-off points with physiological (functional) relevance both in the lower (risk factor) and the upper levels (preventive factor and adverse effects) of reference distribution. This approach should be, in any case, a preliminary and necessary step for any evidence-based decision on the use of lutein supplements in at-risk groups as well as to evaluate the need, adequacy and efficacy of any nutritional interventions with clinical (and economic) impact on disease prevention.

Risk–benefit assessment

Nowadays, the use of supplements (i.e. antioxidant vitamins, lutein-containing supplements) is increasing. This behaviour may stem from the desire to bypass the recommended changes in dietary and lifestyle habits aimed at achieving disease prevention and/or healthy ageing. This behaviour mostly derives from the (assumed) healthy benefits derived from specific nutrients contained in these supplements and the conviction that these compounds are 'natural' and, thus, they are 'safe'. However, simultaneously, the growing concern about their safety has provoked the development of methods for risk assessment and the establishment of safe levels of intake (National Academy of Sciences Institute of Medicine, 2000; Lindsay, 2002).

In any intervention, the benefits should outweigh the risks. However, contrary to non-dietary compounds (i.e. drugs), evaluation of risks with nutrients and food components is compromised by factors related to the nutrient (lack of information on nutrient metabolism, different bioavailability depending on the matrix, interaction with other nutrients) and the subjects (nutritional status, between-subject variability, presence of other risk factors, applicability to gender and age groups, etc.). Risk characterization associated with lutein intake and supplementation is difficult because of the lack of well-controlled large-scale supplementation trials, lack of homogeneity of the studies (variability in sources used, doses, protocols, endpoints and groups assessed), lack of dose-response studies (Lindsay, 2002) and suitable animal models (Lee *et al.* 1999).

However, as with β-carotene, an approximation can be made using serum concentrations. In Fig. 2, ranges of lutein concentrations in serum in control groups with different dietary habits are shown (Olmedilla *et al.* 2001*b*; National Academy of Sciences Institute of Medicine 2000; F Granado, B Olmedilla and I Blanco, unpublished results). Also shown are the ranges achieved under different protocols of lutein supplementation (Olmedilla *et al.* 2002) and those associated with functional effects (blockage of intima-media thickness progression, a biomarker of CVD; Dwyer *et al.* 2001) and improvement of visual acuity (Olmedilla *et al.* 2001*a*). Fig. 2 also shows lutein concentrations achieved in serum in a group of apparently healthy non-smokers supplemented with lutein (15 mg/d for 4 months; Olmedilla *et al.* 2002). In this study, the (reversible) presence of lutein esters in serum in those subjects reaching serum levels above 1·05 μmol/l (> 600 μg/l) was consistently observed (Granado *et al.* 1998). This was an unexpected finding given that lutein circulates in the free form and that carotenoid esters have not been observed with lycopene or α- + β-carotene supplementation at the same doses (Olmedilla *et al.* 2002). These ester forms were present regardless of the total carotenoid concentrations reached in serum, and appeared in serum before and independently of the presence of carotenodermia (clinical sign) and were not related to any change in biochemical or haematological parameters of the subjects and they disappeared upon discontinuation of lutein (Granado *et al.* 1998). It must be noted, however, that the lutein esters represented 3 % of lutein levels achieved in serum. Therefore, the clinical significance could be questionable. Interestingly, in another study using the same capsules but three times/week for more that 2 years (Fig. 2, cataracts + lutein), levels of lutein in serum reached and remained at values of between 0·6 and 1·0 μmol/l without the appearance of ester forms, carotenodermia or changes in the haematological or biochemical profile of the subjects (Olmedilla *et al.* 2001*a*, 2003).

At present, there is almost a complete lack of information regarding the short- and long-term potential adverse and/or toxic effects derived from a high consumption (by supplementation) of most carotenoids, including lutein. The selective accumulation of lutein in patients with systemic amyloidosis (Bruch-Gerharz *et al.* 2001) and the reduced mobilization of retinoids from the liver upon lutein supplementation reported in rats (Jenkins *et al.* 2001) must be taken into account, but should be considered as preliminary findings to be confirmed. The premise that these compounds are safe when consumed at levels above those provided by dietary means is unsubstantiated. In fact, it should not be forgotten that this premise ('safety') was also assumed in the 1980s when large intervention trials using α-tocopherol and β-carotene were initiated, and the results of these studies have demonstrated that this assumption was false, at least for certain risk groups (smokers, alcohol drinkers, asbestos workers) (Albanes 1999; Kushi 1999).

Lutein, in the same way as other phytochemicals, may display a positive risk-benefit ratio depending on the dose and response elicited and the cumulative effect when consumed on a long-term basis (Omenn, 1998; Lindsay, 2002). In addition, it should be considered that when supplied orally, it will have a systemic distribution and that the deposition and the biological effect (i.e. gene expression, apoptosis) in remote tissues is, at present, uncertain and needs to be determined (Collins, 2001). This is especially relevant for lutein, taking into account its selective distribution in certain tissues (i.e. eye), the presence of biologically active oxidation products in serum and tissues, the small but unusual transport forms under non-dietary conditions (i.e. ester forms) and its higher potential for provoking carotenodermia compared with other carotenoids (Olmedilla *et al.* 2002). At present, the factors determining the impact of lutein supplementation in remote tissues are unknown and may be related to the target group, including subject variability (i.e. genetic susceptibility), clinical condition and/or the stage of the

disease. This lack of knowledge and the variability in response translates into uncertainty when considering the potential impact of intervention on a community level.

Approaching cut-off points with physiological relevance

At present, there is no widely accepted 'normal' or reference range for lutein in serum, although some attempts have been made in certain populations (Thurnham, 1988; Ito *et al.* 1990; Sharpless & Duewer, 1995; Olmedilla *et al.* 1997*b*, 2001*b*; National Academy of Sciences Institute of Medicine, 2000). As shown in Fig. 2, concentrations of lutein show wide distributions within and between populations. However, the wider the distribution of an analyte in a population is the greater the number of intervals that can be defined as having potential physiological significance (Sharpless & Duewer, 1995).

In this respect, evidence from both epidemiological and supplementation studies may provide valuable data. While for the lower range of concentrations it is difficult to reach any consensus on cut-off points with functional significance, in the upper part of the distribution the possibility of defining them seems to be more promising, especially considering the potential impact on a community level. Despite the analytical variability and the differences among populations, concentrations $>0{\cdot}60$ μmol/l (>340 μg/l) are consistently at or above the 95th percentile for the lutein distribution in populations with different dietary habits (Fig. 2), as well as those associated with lower levels of oxidative markers (Collins *et al.* 1998*a*,*b*) and lower risk for chronic diseases in epidemiological studies (Dwyer *et al.* 2001). Similarly, short- and long-term studies using different protocols, doses and sources of lutein (diet and capsules) have shown supplementation to be effective in increasing serum concentrations above these levels and in maintaining serum lutein concentrations above the 95th percentile of a reference population (Hammond *et al.* 1997; Landrum *et al.* 1997; Johnson *et al.* 2000; Olmedilla *et al.* 2001*a*). In addition, serum concentrations achieved in these studies ($>0{\cdot}60$ μmol/l) are also consistent with those associated with lower risk for ARMD and cataracts (Eye Disease Case-Control Study Group 1993), with an increase in MPOD upon lutein supplementation (Hammond *et al.* 1997; Landrum *et al.* 1997; Johnson *et al.* 2000) and with an improvement in visual performance in subjects with compromised visual function (Olmedilla *et al.* 2001*a*).

Thus, concentrations of lutein in serum above $0{\cdot}6$ μmol/l (>340 μg/l), achievable by diet, seem to be consistently associated with lower risks in epidemiological studies and lower levels of different biochemical and intermediate markers (i.e. DNA damage biomarkers, reduced intima-media thickness progression, higher MPOD), as well as with an improvement in physiological function (i.e. visual performance) and, thus, quality of life of the subjects. On the other hand, based on the available evidence, serum levels of lutein of up to $1{\cdot}05$ μmol/l (<600 μg/l) seem to be indicative of 'safety' since they are not associated with an unusual serum lutein profile, biochemical or haematological changes or carotenodermia. Thus, concentrations of lutein in serum between $0{\cdot}6$ and $1{\cdot}05$ μmol/l could be considered an achievable and desirable 'biochemical target' on a community level, probably associated with improved health outcomes. These proposed cut-off points, however, must be considered as 'tentative', testable and applicable starting points to be confirmed and validated in relation to different physiological and clinical endpoints, as well as on a community level for disease prevention (i.e. prevention of early atherosclerosis, promotion of visual health).

Nutritional strategies for action

Due to the insufficient evidence and the lack of nutrient specificity (lutein), especially in relation to prevention of cancer and CVD, a food-based rather than a compound-based approach is recommended. Translation of these guidelines for the general population means encouraging a balanced diet rich in a variety of fruits and vegetables, including green and yellow. Since the effect of diet is cumulative, this is a prudent and safe approach that will simultaneously provide other phytochemicals with potential positive effects in disease prevention.

Available dietary strategies to increase lutein intake in the population are shown in Table 5. As can be seen, all of them could be compatible within a balanced diet and achievable at a relatively low cost. In addition, some of them are safe and applicable on a community basis, whereas other dietary approaches could be of interest and helpful in at-risk groups, once their safety had been proven. In this respect, it should also be considered, however, that in a proportion of subjects at high risk for developing such diseases (i.e. genetic predisposition), presenting

Table 5. Dietary strategies to increase lutein intake

Strategy	Balanced diet	Applicability	Safety	Cost
To encourage consumption of lutein-rich fruit and vegetables	Compatible	Public health	Yes	Low
To enhance lutein content in foods (traditional breeding techniques or plant biotechnology) through 'Biofortification'	Compatible	Public health Groups at risk	Yes To be proved	Medium–Low
To optimize industrial processing to increase lutein retention and bioavailability	Compatible	Public health	Yes	Low
Functional foods (fortification and supplementation of foods, newly designed foods)	Compatible	Groups at risk	To be proved	Low
Use of lutein-rich natural extracts or lutein supplements	Compatible	High-risk groups	To be proved	Low

special nutritional demands (i.e. malabsorption syndromes) and/or having some pathological eye disease compromising visual function (i.e. retinitis pigmentosa, cataracts, ARMD), the use of 'biofortification' and/or lutein supplements, within a balanced diet, would probably help to ameliorate the disability and improve the quality of life. Some of these subjects at high risk could be considered a 'target' group for immediate intervention (i.e. elderly individuals, subjects at initial stages of macular degeneration, and/or cataracts).

In considering the use of lutein supplements in disease prevention (i.e. CVD, age-related eye disease), decisions should be based on the available evidence and the potential risks should be evaluated, keeping in mind the unexpected results from previous intervention trials in human subjects using single nutrients (i.e. β-carotene). However, the variability in response to lutein supplementation suggests that, in terms of applicability on a community basis, efficacy of intervention would have to be considered in terms of probability, in addition to safety and cost savings. In any case, well-designed, randomized clinical trials are needed to evaluate the benefits and risks of supplementation with lutein in relation to human health and disease and, until such effects are proven and safety established, the indiscriminate use of lutein supplements cannot be justified.

Final considerations

Despite the increasing amount of literature dealing with different aspects of lutein in man, our aim was to briefly review the available evidence and provide, within the context of evidence-based medicine, some rationale for the decision-making process regarding the potential use of lutein in clinical practice and public health.

At present, there are no definitively established, physiologically significant cut-off points for lutein in serum above which 'protection' or prevention against chronic diseases is ensured or provided. Similarly, there are no recommendations for optimal nutritional intake of lutein. There is little available information on lutein supplementation in human consumers. No extra benefits or harmful effects in terms of the onset or progression of chronic and degenerative diseases in apparently healthy subjects have been demonstrated. Nevertheless, available evidence suggests that serum concentrations of lutein in the range of 0·6–1·05 μmol/l (350–600 μg/l) may be achievable by dietary means and could be considered a potential 'target' for health promotion and disease prevention. Although this range of serum concentrations may be considered too 'cautious', in concentrations of lutein above this cut-off point (>1·05 μmol/l), unusual lutein ester forms appear in plasma and have not been associated with extra benefits with regard to different oxidative markers related to chronic diseases. In this respect, there should be awareness that, similar to the case of β-carotene, the range between benefits and harm may be narrow (Van den Berg *et al.* 2000) and, thus, there should be caution when establishing recommendations for lutein intake above levels achievable by dietary means. The percentage of the population consuming lutein-containing supplements is increasing and a variety of lutein-fortified and supplemented foods could become commercially available (Surai *et al.* 2000). The consumption of these products is frequently driven by the publicity regarding 'unproven properties' of this compound, and is even recommended by physicians who, in the absence of data about the efficacy of the treatment and the apparently lack of adverse effects, make an intuitive decision based on the ethical principle of beneficence (Guerra-Romero, 1996), rather than following the principles of evidence-based medicine. The claims often state that lutein is safe merely on the basis of the lack of adverse effects reported to date. There is at least one argument to refute this assertion: the lack of reports of significant adverse or side effects to date (that is, the lack of knowledge) is not, in essence, a proof of safety. This 'safety' should be considered, at least, unsubstantiated and based only on the lack of evidence, since no specific studies have been performed to test it and lutein has not been used on a large scale, in groups with different characteristics, at different doses or for a sufficient time to provoke any significant and measurable potential adverse effect. This, in fact, reflects two different approaches in public health, that is, to prove that a compound is unsafe rather than to prove it is safe for human health.

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