Food Chemistry 45 (1992) 205–213
Analytical Methods Section

Determination of nine carotenoids, retinol, retinyl palmitate and α-tocopherol in control human serum using two internal standards

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(Received 16 January 1991; revised version received 17 May 1991; accepted 20 May 1991)

In the determination of the carotenoids in 39 samples of human serum from Spanish control subjects, those most abundant were found to be β-cryptoxanthin, lycopene, lutein and β-carotene; the least abundant carotenoids were zeaxanthin and α-carotene. Lutein and zeaxanthin, when analysed separately, were present at a ratio of about 3:1 in all the sera studied. One xanthophyll, identified as canthaxanthin, was detected in all serum samples. Other carotenoids, such as γ-carotene and β-apo-8'-carotenal, were found in only some of the samples. Retinol and α-tocopherol were determined simultaneously, as was the tocopherol/cholesterol ratio. Retinyl palmitate was measured in only one of the control subjects, although its elution produced no interference with β-carotene or *cis*-β-carotene. As internal standards, retinyl acetate was used for retinol and tocopherol acetate for α-tocopherol and the carotenoids.

INTRODUCTION

In-vitro studies of some retinoids, carotenoids and tocopherol have shown their effectiveness against certain types of cancer (Krinsky & Deneke, 1982; Ames, 1983). Although some of them are found to be present in the human diet, it is difficult to extrapolate directly from chemical to biological systems. In spite of this fact, there are numerous epidemiological studies which demonstrate significant correlations between the ingestion of certain foods and the prevention of some types of cancer, cardiovascular diseases and some chronic disorders (Shekelle *et al.*, 1981; Weber & Goerz, 1986; Block, 1989; Cutler, 1989). Moreover, other constituents of foods rich in carotenoids, such as fibre, ascorbic acid, vitamin E, calcium, selenium, indoles and polyphenols, are also plausible protective factors against cancer and other diseases (Willett, 1989; Ziegler, 1989).

There are carotenoids other than β-carotene which are also effective antioxidants, depending on the site of their activity, making it equally important to monitor their presence in serum. Like β-carotene, which has been epidemiologically associated with cancer, there are similar studies dealing with lycopene, lutein, canthaxanthin and phytoene (Mathews-Roth, 1982; Burney *et al.*, 1989; Krinsky, 1989; Le Marchand *et al.*, 1989).

However, in spite of the evidence, the hypothesis of a significant protective role for such substances remains promising, but unproved, since it must be taken into account that, *in vivo*, most of them act synergically and thus should not be considered separately with regard to their role in the prevention of certain diseases (Bendich & Olson, 1989; Gey, 1989; Le Marchand *et al.*, 1989).

The multifactorial nature of the events that can initiate diverse pathological processes and of the intervention of possible protective substances in the organism lends great interest to the analytical study of the largest possible number of the implicated compounds. For this purpose, the authors have undertaken the determination of the levels of the liposoluble vitamins, *trans*-retinol and D-α-tocopherol (and the tocopherol/cholesterol ratio), as well as the different carotenoids present in the serum of control subjects. Figure 1 shows the structure of the different compounds dealt with in this study.

MATERIALS AND METHODS

Reagents

Lycopene, all-*trans*-α- and β-carotene, all-*trans*-retinol, retinyl acetate, retinyl palmitate, D-α-tocopherol and

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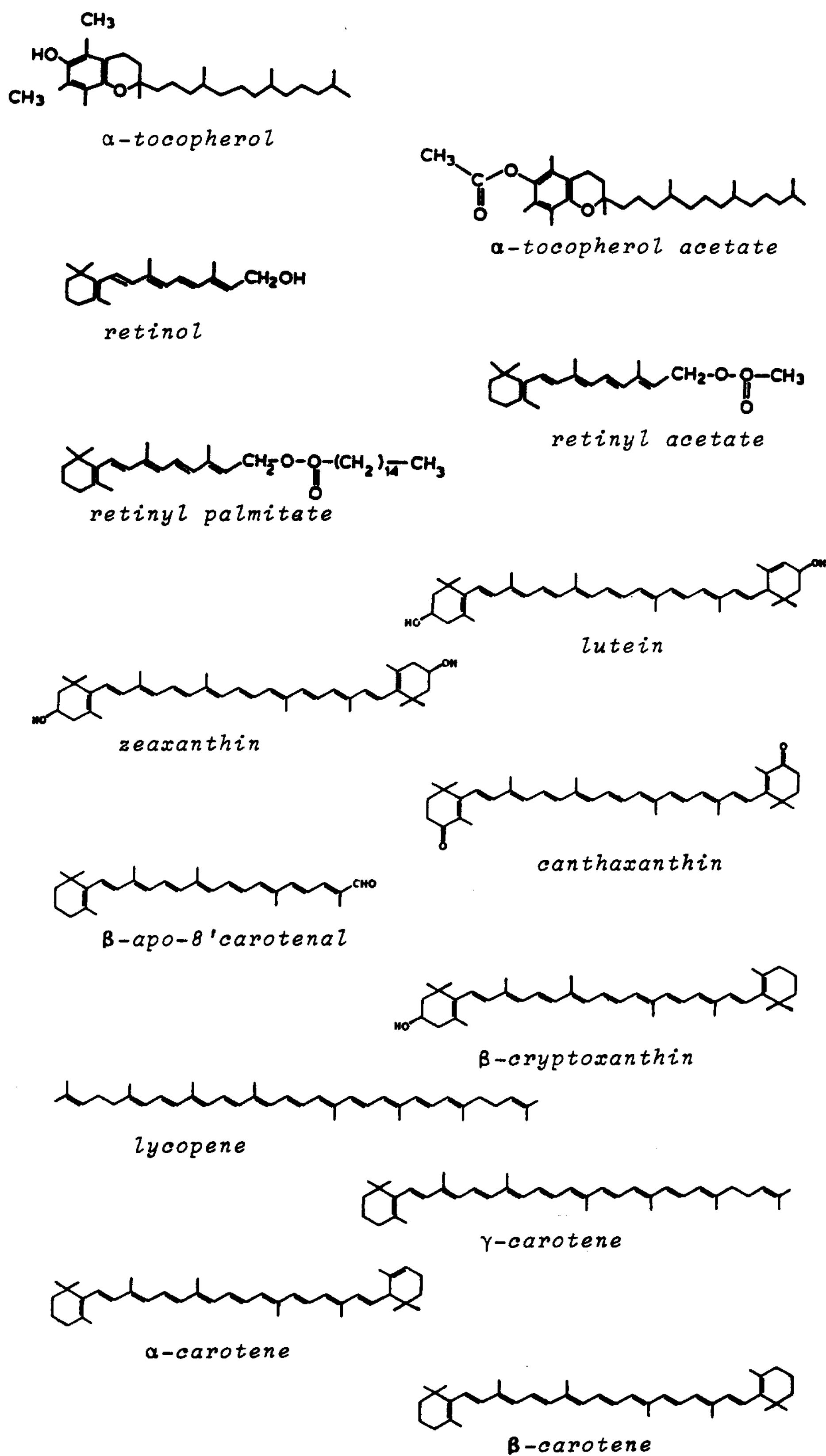

Fig. 1. Chemical structure of the compounds dealt with in this study.

D-α-tocopherol acetate standards were obtained from Sigma Chemical Co. (St Louis, MO). β-Apo-8'-carotenal was obtained from Fluka Chemie AG (Buchs, Switzerland). γ-Carotene, canthaxanthin, β-cryptoxanthin, 15-*cis*-β-carotene, lutein and zeaxanthin were gifts from Hoffmann-La Roche (Basel, Switzerland). Tetrahydrofuran (THF) was obtained from Scharlau (Barcelona, Spain). Hydroxybutyltoluene (BHT) and dichloromethane were purchased from Carlo Erba (Barcelona, Spain). Methanol, ethanol, *n*-hexane and acetonitrile (HPLC grade) were supplied by Merck (Spain).

Equipment

The HPLC system consisted of an ALC/GPC chromatograph (model 201, Waters Associates, Milford, MA) equipped with a model 6000 A pump, dual reciprocating piston heads, model U6K septumless injector and programmable multiwavelength detector, model 490 E (Waters Associates). The detector signals were recorded on a M730 data module (Waters Associates) and an Omniscribe recorder. The spectrophotometer employed for this study was an Uvicon 930 from Kontron.

Chromatographic conditions

As described in detail previously (Olmedilla *et al.*, 1990), two different solvent systems were used for the reversed phase quantification of the serum samples as follows:

—System I: a 5 μm Spheri-5-RP-18 column (Brownlee Labs, Applied Biosystem, Santa Clara, CA, USA), 220 mm × 4·6 mm, with a guard column of Aquapore ODS type RP-18, 15 mm × 3·2 mm, 7 μm. Solvent, acetonitrile/dichloromethane/methanol (70 : 20 : 10, v/v/v); flow rate, 1·8 ml min^{-1}; 5 μl was injected. Channel 1, detection at 450 nm: lutein + zeaxanthin, β-cryptoxanthin, lycopene, γ-carotene, α-carotene and β-carotene. Channel 2, detection at 313 nm (retinol and retinyl acetate), changing at min 3 to 294 nm (α-tocopherol, α-tocopherol acetate), and at min 6·5 to 325 nm (retinyl palmitate).

—System II: column and precolumn as in system I. The mobile phase, acetonitrile/methanol (85 : 15, v/v); flow rate, 1·8 ml min^{-1} ; 5 μl was injected. Detection at 450 nm: lutein, zeaxanthin, canthaxanthin, β-apo-8'-carotenal.

The minimum quantifiable amounts for each component, expressed in μg dl^{-1} serum, in system I are: 1.2 μg dl^{-1} for lutein, cryptoxanthin and α-carotene, as well as retinol; 3·8 μg dl^{-1} for lycopene and β-carotene; 9 μg dl^{-1} for retinyl palmitate; and 57 μg dl^{-1} for tocopherol. In system II, they are: 2 μg dl^{-1} for lutein and zeaxanthin and 1 μg dl^{-1} for canthaxanthin and β-apo-8'-carotenal.

Sample preparation

Forty-three healthy volunteers (14 males and 29 females; age range 20–57 years, but predominantly 20–40 years (median age, 33); mostly medical students and clinical staff of this hospital) participated in the study.

Serum samples were collected at 9.00 a.m. after overnight fasting. They were analysed in duplicate on the same day or were frozen and stored at −20°C until used (at most, one month later). All procedures were performed in subdued light.

To serum samples (800 μl) were added ethanol containing α-tocopherol acetate and retinyl acetate as internal standards (800 μl), and ethanol (800 μl), or water in the case of the standard curve for retinol, retinyl palmitate and tocopherol. After being vortexed for 45 s, they were extracted twice with hexane (2 ml, stabilized with BHT) and vortexed for 3 and 2 min, respectively. The hexane supernatants were removed and pooled. Aliquots (3·5 ml) were taken, evaporated *in vacuo*, reconstituted with THF (0·3 ml, stabilized with BHT) and vortexed for 45 s. Five microlitres of this solution were injected under the conditions used in system I and 0·1 ml was separated, evaporated to dryness *in vacuo* and reconstituted with THF/ethanol (1 : 3, v/v), to be injected into the chromatograph using the mobile phase described for system II.

Evaluation of the serum concentration of each carotenoid was based on a standard curve containing all the carotenoids to be quantified in each of the two systems described, using a THF solution (containing 0·01% BHT) of the following carotenoids (approximate concentrations prior to spectrophotometric correction): lutein, 1–3 μg ml^{-1}; cryptoxanthin, 1–3 μg ml^{-1}; lycopene, 1–2 μg ml^{-1}; γ-carotene, 0·4 μg ml^{-1}; α-carotene, 0·4–1·3 μg ml^{-1}; β-carotene, 1–2 μg ml^{-1}; and tocopherol acetate, 0·3 mg ml^{-1}. This solution was prepared daily and diluted to create an external standard curve (height units versus concentration of the carotenoid injected); 5 μl were injected.

For the analysis of carotenoids in system II, another standard curve was prepared in ethanol. The approximate concentrations prior to spectrophotometric correction were: lutein, 1–3 μg ml^{-1}; zeaxanthin, 0·5–1 μg ml^{-1}; canthaxanthin, 0·4 μg ml^{-1}; β-apo-8'-carotenal, 0·4 μg ml^{-1}; and tocopherol acetate, 0·3 mg ml^{-1}.

Serum concentrations of retinol, retinyl palmitate and tocopherol were based on a standard curve containing retinyl acetate and tocopherol acetate as internal standards. This curve was extracted twice, as for the serum samples. The approximate concentrations of these compounds were: retinol, 1 mg ml^{-1}; retinyl

Table 1. Spectrophotometric characteristics of retinoids, tocopherols, and carotenoids used to standardize the assay

Substance	Solvent	Wavelength (nm)	*E* 1% 1 cm	Reference
Retinol	EtOH	325	1835	Merck Index (1983)
Retinyl acetate	EtOH	326	1550	Merck Index (1983)
Retinyl palmitate	EtOH	328	975	Merck Index (1983)
D-α-Tocopherol	EtOH	292	72	Machlin (1984)
D-α-Tocopherol acetate	EtOH	285	40	Machlin (1984)
Lutein	EtOH	445	2550	De Ritter & Purcell (1981)
Zeaxanthin	EtOH	450	2540	De Ritter & Purcell (1981)
Canthaxanthin	PE	466	2200	De Ritter & Purcell (1981)
β-Apo-8'-carotenal	PE	457	2640	De Ritter & Purcell (1981)
β-Cryptoxanthin	PE	452	2386	De Ritter & Purcell (1981)
Lycopene	PE	472	3450	De Ritter & Purcell (1981)
γ-Carotene	Hexane	462	3100	De Ritter & Purcell (1981)
α-Carotene	PE	444	2800	De Ritter & Purcell (1981)
β-Carotene	PE	453	2592	De Ritter & Purcell (1981)

PE, Petroleum ether.

acetate, 0·6 mg ml^{-1}; tocopherol, 3·5 mg ml^{-1}; tocopherol acetate, 0·1 mg ml^{-1}; and retinyl palmitate, 1 mg ml^{-1}

The mean (± standard deviation) recoveries of exogenous retinol and α-tocopherol were 96 ± 8 and 98 ± 9% (n = 11), respectively. Carotenoid recoveries were not performed.

System calibration and assay

Stock solutions of each carotenoid were prepared by dissolving 1–3 mg of the compound in 50 ml of THF containing BHT (0·01%). Standards were stored at –20°C and protected from light. These solutions were further diluted to provide working standards.

Stock solutions of retinoids, D-α-tocopherol and D-α-tocopherol acetate were prepared by dissolving different amounts in ethanol to obtain concentrations ranging from 0·05 to 1 mg ml^{-1}.

The purity of available standards (commercial or not) was unpredictable and varied with the lot purchased. Concentrations were calculated by correcting the amounts weighed on the basis of published absorptivity values (*E* 1% 1 cm) as indicated in Table 1.

Solutions were discarded when spectrophotometric checks revealed a drop in concentration, and, in any case, a complete mixture of standard compounds was prepared monthly.

Injection of the standard curve was alternated with that of the sample during the chromatographic session. The correlation coefficient between the height of each peak and the concentration of the corresponding compound in the standard solution ranged 0·990–0·999.

Peak identification was confirmed by comparing relative plasma peak retention times with those of known standards, by demonstrating coelution of plasma peaks and known standards added to the serum matrix, by

Table 2. Variability of retinol, tocopherol and carotenoid analysis of control serum

Analyte	Within same day		Between days	
	Mean* ± SD	CV (%)	Mean** ± SD	CV (%)
Retinol	2·0 ± 0·02	0·8	2·0 ± 0·04	1·4
α-Tocopherol	34·8 ± 0·7	1·8	34·8 ± 0·9	2·6
Lutein	10·7 ± 0·4	4·2	10·5 ± 0·5	4·7
Zeaxanthin	2·7 ± 0·03	1·2	2·6 ± 0·15	5·7
Canthaxanthin	1·2 ± 0·03	10·4	1·3 ± 0·2	14·6
β-Cryptoxanthin	23·8 ± 0·7	2·3	25·8 ± 2·1	8·0
Lycopene	31·7 ± 1·2	3·7	28·7 ± 2·3	7·9
α-Carotene	2·7 ± 0·3	10·9	2·9 ± 0·4	11·9
β-Carotene	17·9 ± 0·7	3·8	18·9 ± 1·2	6·4

*Mean of six samples.
**Mean of six duplicate assays.
The means for retinol and α-tocopherol are expressed in μmol $litre^{-1}$.
The means for carotenoids are expressed in μg dl^{-1}.

monitoring the UV absorbance ratios of the peaks and by stop-flow scan.

To assess the precision of the method, a pooled serum sample was analysed six times on the same day, and the same pooled sample was extracted and analysed six times on two consecutive days (Table 2). The coefficients of variation (CV) for α-carotene and canthaxanthin, both within the same day and between days, were greater than those usually obtained because of the low concentration of the two compounds in the pool analysed when comparing with the mean for the population studied. The authors did not obtain the CVs for β-apo-8'-carotenal and γ-carotene as these compounds were not present in the pool.

RESULTS AND DISCUSSION

Most reports have focused on the hydrocarbon carotenoids (lycopene, α-carotene and β-carotene), while there are fewer dealing with the more polar xanthophyll pigments (Parker, 1989) such as lutein and zeaxanthin. Carotenoids present in the human diet and detectable in serum include β-carotene, α-carotene, cryptoxanthin, lycopene and lutein as major components, with smaller concentrations of zeaxanthin, other xanthophylls and polyenes such as phytofluene and phytoene (Bendich & Olson, 1989).

Using the previously reported method (Olmedilla *et al.*, 1990), the authors determined, aside from the carotenoids considered to be major components in serum, others which appear in smaller amounts such as zeaxanthin, canthaxanthin, β-apo-8'-carotenal and γ-carotene, which are found both in certain foods and food additives (Bauernfeind, 1981).

In serum analysis, the use of an internal standard allows better quality control and helps correct for factors such as evaporation of the reconstituted sample. As Bieri *et al.* (1985) indicate, the recovery of carotenoids added to serum can be misleading since those added in a solvent are more readily extractable than the lipoprotein-bound carotenoids in serum. For this reason, the authors' initial extractions were done with tocopherol acetate as internal standard for the carotenoids and tocopherol because it elutes halfway between a wide range of very polar to nonpolar carotenoids. Retinyl acetate was used as an internal standard for retinol.

The authors examined echinenone as an internal standard because it elutes between β-cryptoxanthin and lycopene; it was found, in contrast with reports by other authors (Cantilena & Nierenberg, 1989), that it does not interfere with any of the carotenoids analysed (Bieri *et al.*, 1985). However, since both tocopherol acetate and echinenone elute in the same zone of the chromatogram, as an internal standard the authors used the former with which they had previously obtained good recoveries, in agreement with other authors (Cavina *et al.*, 1988; Thurnham *et al.*, 1988). On the other hand, the use of echinenone as an internal standard may be a problem when the sera being analysed are from populations that consume food rich in this compound, such as sea urchins and their by-products.

Figures 2 and 3 show the chromatographic profiles corresponding to serum samples analysed in the conditions referred to here as systems I and II, respectively.

Retinyl palmitate does not interfere with β-carotene or with the possible presence of isomers detected at the tail of the peak and absorbed at 325 nm, as can be observed in Fig. 2. The ratio 340 : 450 nm has a value greater than 0·20 at the tail of the β-carotene peak

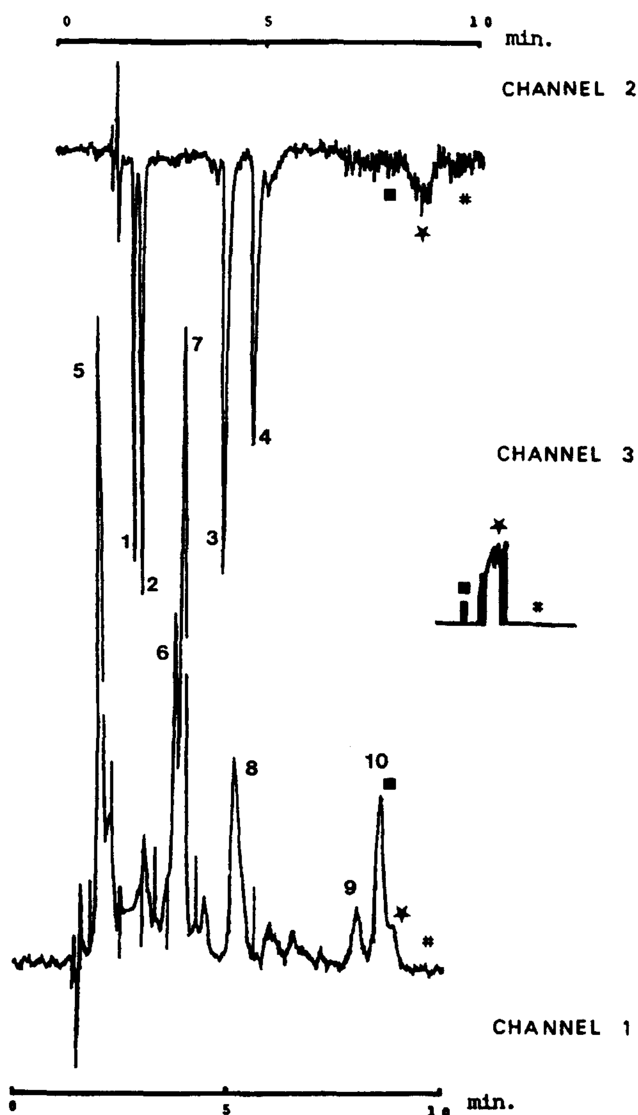

Fig. 2. Chromatogram of a serum extract. Chromatographic conditions are described in the text (system I). Chart speed: 0·7 cm min^{-1}. Channel 1: detection 450 nm, 0·005 AUFS. Channel 2: detection 313–294 nm (0·01 AUFS), 325 nm (0·003 AUFS). Channel 3: ratio 340:450 nm (0·003 AUFS). (1) Retinol; (2) retinyl acetate (internal standard); (3) α-tocopherol; (4) α-tocopherol acetate; (5) lutein and zeaxanthin; (6) pre-β-cryptoxanthin; (7) β-cryptoxanthin; (8) lycopene; (9) α-carotene; (10) β-carotene. Areas corresponding to β-carotene (■), *cis*-β-carotene (★) and retinyl palmitate (#) in each of the three channels, respectively.

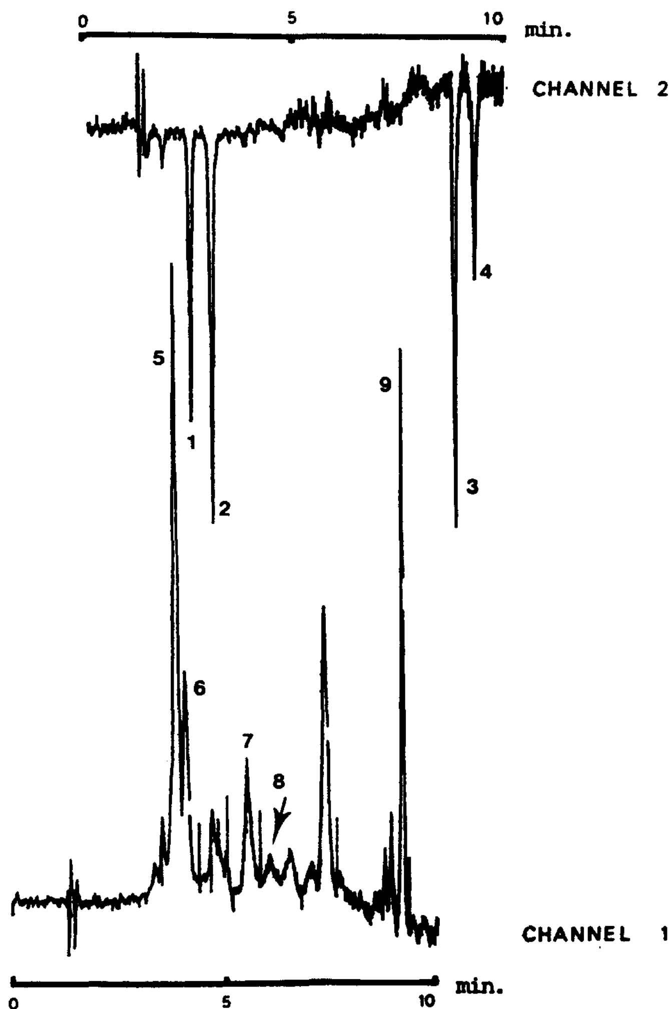

Fig. 3. Chromatogram of a serum extract. Chromatographic conditions are described in the text (system II). Chart speed: 0·7 cm min⁻¹, at 9 min, 0·35 cm min⁻¹. Channel 1: detection 450 nm, 0·005 AUFS; change to 0·003 at 5 min. Channel 2: detection 313 nm (0·01 AUFS); 294 nm (0·005 AUFS). (1) Retinol; (2) retinyl acetate (internal standard); (3) α-tocopherol; (4) α-tocopherol acetate (internal standard); (5) lutein; (6) zeaxanthin; (7) canthaxanthin; (8) β-apo-8'-carotenal (arrow); (9) cryptoxanthin.

(indicative of *cis*-β-carotene), while at the top of the peak, the value of the ratio is less than 0·20 (which corresponds to all-*trans*-β-carotene). In the area corresponding to retinyl palmitate, no reading is obtained for this ratio (Fig. 2, channel 3).

The authors have detected the possible presence of *cis*-carotenoids in serum samples, as reported by Bieri *et al.* (1985) and Sowell *et al.* (1988), at the downslope of the peaks corresponding to lycopene and β-carotene.

The values obtained in the analysis of the control sera are shown in Table 3, expressed as the means obtained for the entire group of subjects and for the groups corresponding to males and females separately.

The retinyl palmitate levels are not given in Table 3 because this substance could only be measured in one of the controls (11 μg dl^{-1}).

The retinol levels obtained are within the range 1·1–3·0 μmol $litre^{-1}$. The mean value for retinol in males is greater than that found for females, the opposite of what occurs in the case of carotenoids, as has been reported earlier by some authors (Olson, 1984; Stacewicz-Sapuntzakis *et al.*, 1987; Krasinski *et al.*, 1989).

Regarding serum α-tocopherol levels, no differences are observed between those of males and females, all of which fall within the range 21–54 μmol $litre^{-1}$. On the other hand, serum tocopherol is highly correlated with cholesterol levels (Thurnham *et al.*, 1986), and when the tocopherol/cholesterol ratio is established, the difference between two populations studied by Thurnham (1990) disappears.

Thurnham (1990) obtains values for the tocopherol/cholesterol ratio of 5·35 μmol $mmol^{-1}$ in the Chinese population and 4·65 μmol $mmol^{-1}$ for controls from the UK. In Spanish subjects, Fernández-Banares *et al.* (1989) report a value of 6·1 mg g^{-1} (5·52 μmol $mmol^{-1}$) for this ratio. In the population studied by the present authors, the value is 6·3 ± 1·2 μmol $mmol^{-1}$ (n = 35), which is slightly higher than those reported elsewhere, with an elevated standard deviation which may be due to the reduced size of the group evaluated to date. Nevertheless, the range for this ratio in the authors' controls is 4·4–8·7 μmol $mmol^{-1}$, which covers all the aforementioned values.

In the literature dealing with the analysis of serum carotenoids in control subjects, we find joint values for lutein + zeaxanthin. This is perhaps due to the inability to separate them for serum analyses—although according to some reports it is possible using standards (Cantilena & Nierenberg, 1989)—or because zeaxanthin is not considered to be a usual component of serum by some authors (Stacewicz-Sapuntzakis *et al.*, 1987) or is detected in very small amounts by others (Kaplan *et al.*, 1987).

The lutein and zeaxanthin present in serum have been quantified jointly using system I and separately with system II. The amount of lutein is always greater than that of zeaxanthin in a proportion of about 3 : 1, which coincides with that mentioned by Baruna *et al.* (1989).

The authors found canthaxanthin, a carotenoid present in its natural form in mushrooms, crustaceans, trout, salmon, etc., and widely used as a food and feed colourant, in all the sera analysed, although in small quantities. Its presence in serum in very small amounts has been mentioned by Kaplan *et al.* (1987) and Stacewicz-Sapuntzakis *et al.* (1987), although they do not specify the concentrations.

The presence of canthaxanthin in serum samples is detectable by both systems I and II, but it is in the latter that this substance has been quantified since it offered a better resolution of the xanthophylls. The spectrum of canthaxanthin presents (in the mobile phase of system II) a maximum between 465 and 477 nm with a shoulder at 420 nm in the standard as well

Table 3. Retinol, tocopherol and carotenoid levels in the sera of control subjects

	Men mean ± SD range ( ) *n*	Women mean ± SD range ( ) *n*	All controls mean ± SD range ( ) *n*	Median all controls
Retinol (μmol litre^{-1})	2·3 ± 0·4 (1·7–3·0) 14	1·8 ± 0·5 (1·1–2·9) 29	2·0 ± 0·5 (1·1–3·0) 43	1·9
D-α-Tocopherol (μmol litre^{-1})	37·2 ± 9·9 (23·5–54·4) 14	34·4 ± 7·5 (20·9–54·1) 29	35·3 ± 8·4 (20·9–54·4) 43	34·6
Tocopherol/cholesterol ratio (μmol mmol^{-1})	6·4 ± 1·5 (4·4–8·4) 12	6·3 ± 1·1 (4·5–8·7) 23	6·3 ± 1·2 (4·4–8·7) 35	6·4
Lutein + zeaxanthin	20·7 ± 9·0 (8·6–39·3) 12	21·7 ± 9·8 8·6–52·9) 27	21·4 ± 9·5 (8·6–52·9) 39	19·1
Lutein	14·5 ± 5·0 (6·3–23·7) 12	15·3 ± 7·1 (7·2–42·8) 27	15·1 ± 6·5 (6·3–42·8) 39	13·7
Zeaxanthin	4·3 ± 2·0 (1·5–8·2) 12	4·6 ± 2·4 (1·5–10·2) 27	4·5 ± 2·2 (1·5–10·2) 39	4·1
Canthaxanthin	1·9 ± 1·0 (0·8–3·4) 12	2·1 ± 1·1 (0·9–4·8) 27	2·0 ± 1·0 (0·8–4·8) 39	1·7
β-Apo-8'-carotenal[a]	1·1 ± 0·5 (0·4–1·7) 5	1·4 ± 0·5 (0·9–2·2) 9	1·3 ± 0·5 (0·4–2·2) 14	1·3
β-Cryptoxanthin	17·5 ± 11·2 (6·2–47·7) 12	32·3 ± 25·3 (5·2–112·1) 27	27·7 ± 22·8 (5·2–112·1) 39	20·8
Lycopene	33·2 ± 16·8 (13·3–63·7) 12	29·6 ± 13·6 (6·8–64·9) 27	30·6 ± 14·5 (6·8–64·9) 39	28·3
γ-Carotene[a]	5·6 ± 1·1 (4·4–6·1) 3	4·4 ± 2·9 (1·5–9·0) 8	4·8 ± 2·5 (1·5–9·0) 11	5·0
α-Carotene	4·2 ± 2·0 (2·1–7·5) 11	4·4 ± 2·4 (0·7–11·6) 27	4·3 ± 2·3 (0·7–11·6) 38	3·9
β-Carotene	13·9 ± 5·5 (7·6–24·0) 12	19·6 ± 9·0 (5·2–44·1) 27	17·9 ± 8·4 (5·2–44·1) 39	16·9
Total carotenoids quantified	89·5 ± 33·0 (60·3–167·7)	109·2 ± 45·7 (46·0–229·0)	103 ± 42·8 (46·0–229·0)	92·2

All carotenoids are expressed as μg dl^{-1}.
[a] Mean values correspond to the quantified sera. These samples in which a given carotenoid was not detected are not included in the calculation of the mean.

as in the sample. A xanthophyll with similar spectral characteristics that elutes in the same zone of the chromatogram, between lutein–zeaxanthin and β-cryptoxanthin, has been described by Bieri *et al.* (1985), although they do not identify it as canthaxanthin.

Like canthaxanthin, β-apo-8'-carotenal is used as a food colourant and is found in nature in green plants and citrus fruit. However, its presence in the serum samples (14 out of 39 analysed) was not uniform as that of canthaxanthin, and it appeared in smaller amounts.

Likewise, γ-carotene has only been quantified in some of the control sera. It was detected in 11 of the 39 samples assessed for carotenoids in the range 1·5–9·0 μg dl^{-1}. This range was similar to that obtained for α-carotene, although the latter was invariably found in all the sera analysed.

Like lutein and zeaxanthin, β-cryptoxanthin, lycopene, α-carotene and canthaxanthin have been found in all the samples. Of these, lycopene and β-cryptoxanthin are the most abundant, while zeaxanthin, α-carotene (with similar concentrations) and canthaxanthin are the least abundant.

The presence of a peak immediately before β-crypto-

Table 4. Serum retinol, α-tocopherol (μmol litre⁻¹) and carotenoid (μg dl⁻¹) concentrations in this study and in three previous reports (mean ± SD)

Analyte	Present study	Cavina *et al.* (1988)	Stacewicz-Sapuntzakis *et al.* (1987)	Cantilena & Nierenberg (1989)
Retinol	2·0 ± 0·5	2·3 ± 0·8	2·4 ± 0·7	
α-Tocopherol	35·3 ± 8·4	29·7 ± 6·9		
Lutein + zeaxanthin	21·4 ± 9·4[a]	28·4 ± 9·1	18·4 ± 8·7	19·8 ± 7·9
Canthaxanthin	2·0 ± 1·0			
β-Apo-8'-carotenal	1·3 ± 0·5[b]			
β-Cryptoxanthin	27·7 ± 22·8	20·0 ± 16·6	9·2 ± 5·6	9·5 ± 4·0
Lycopene	30·6 ± 14·5	39·5 ± 18·3	20·0 ± 12·2	13·9 ± 5·1
γ-Carotene	4·8 ± 2·5[c]			
α-Carotene	4·3 ± 2·3	2·6 ± 1·6	3·4 ± 2·3	4·5 ± 3·4
β-Carotene	17·9 ± 8·4	31·3 ± 17·4	20·3 ± 13·2	13·6 ± 7·9
Σ-Carotene	103·1 ± 42·8	121·4	71·3	61·3
n	39(27F–12M)	14(11F–3M)	110(55F–55M)	33(18F–15M)
Age (years)	20–55	—	49–69	20–41
Origin	Spain	Italy	USA	USA

[a] Lutein = 15·1 ± 6·5, zeaxanthin = 4·5 ± 2·2.
[b] n = 14.
[c] n = 11.
F, female; M, male.

xanthin, designated by Bieri *et al.* (1985) as pre-β-cryptoxanthin, has been detected—although not quantified—in all the sera analysed.

The mean of all the carotenoids quantified is greater in females than in males and corresponds to the differences between the two groups with respect to their β-carotene and β-cryptoxanthin levels. The values for lutein, zeaxanthin, lycopene, canthaxanthin, β-apo-8'-carotenal and α-carotene are similar for the two groups. These observations coincide with data provided by Stacewicz-Sapuntzakis *et al.* (1986).

Table 4 shows the mean concentrations of carotenoids in serum found in the present study, as well as the values reported in other studies carried out in control subjects (Stacewicz-Sapuntzakis *et al.*, 1987; Cavina *et al.*, 1988; Cantilena & Nierenberg, 1989). The sum of the carotenoids quantified in each study is indicated, as well as number of controls analysed, age and geographical region of each.

Although the assessment of average carotenoid levels in healthy populations is subject to fluctuations due to factors such as age, sex, diet and geographical region, some considerations can be established.

The sums of the carotenoids quantified in the studies carried out in Italian and Spanish control subjects are similar and greater than those obtained with controls in the USA. When compared individually, the greatest difference is observed in the β-cryptoxanthin and lycopene levels, the means of which are higher both in the authors' study and in that performed by Cavina *et al.* (1988) with respect to studies carried out in subjects from the USA. The higher β-cryptoxanthin levels in the two studies may be due in part to the larger proportion of females in these groups since, as indicated above, they have higher concentrations of this compound than males.

Regarding the comparison of lycopene levels in American subjects assessed by Cantilena and Nierenberg (1989) (n = 33) and those dealt with here (n = 39), a significantly higher concentration is found in the authors' study ($p \leq 0{\cdot}001$).

The authors report values for β-carotene comparable to those of lutein, the predominant carotenoids in this study being lycopene and β-cryptoxanthin. Nor in the report published by Stacewicz-Sapuntzakis *et al.* (1987) was β-carotene the most prevalent carotenoid in human serum, where it represented 28% of those present. Cavina *et al.* (1988) did not find it to be predominant in serum either, reporting comparably high levels for lycopene and zeaxanthin plus lutein.

Given the fluctuations in carotenoid levels depending on the groups analysed, the authors consider that it will be of interest in the future to broaden the studies of the polar and nonpolar carotenoids in human blood and tissues and to increase the number of populations analysed, classifying them according to age, sex and the season of the year.

ACKNOWLEDGEMENTS

This investigation was partially funded by grant 89/0110 from the Fondo de Investigaciones Sanitarias (FIS), Spain. The authors are indebted to Dr W. Schüep and Dr H. E. Keller of Hoffmann-La Roche, Basel, Switzerland, for supplying the carotenoids. The authors also wish to thank Teresa Motilla and Pilar

Martínez for the plasma collection and Martha Messman for the preparation of the manuscript.

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