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Carotenoid profile and retinol content in human serum – simultaneous determination by high-pressure liquid chromatography (HPLC)

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A reversed-phase HPLC method developed for the simultaneous determination of carotenoids and retinol in foods of vegetable and animal origin was applied to the study of 100 samples of human serum. The subjects were urban adult Malaysians of Malay, Chinese and Indian descent with a mean age of 52.8 years (range 17–78 years). For comparison, all serum samples were also simultaneously determined using the direct spectrophotometric method for carotenoids and the Carr–Price colorimetric method for retinol. Compared to the conventional methods, the HPLC method was found to give significantly higher results for retinol and total carotenoid concentrations. The major advantages of the liquid chromatographic method are that it is more specific and that it overcomes the problems associated with the Carr–Price method. In addition, only the HPLC procedure could provide an account of the serum carotenoid profile, a knowledge of which is now of increasing health importance in view of the possible inverse association of carotenoid intake and some forms of cancer. Six major carotenoids – lutein, cryptoxanthin, lycopene, γ-carotene, α-carotene and β-carotene – were quantitated, the most abundant being lutein and cryptoxanthin, each contributing to about one quarter of all the carotenoids quantitated. β-carotene and lycopene were the next abundant carotenoids, each contributing to about 20% of all carotenoids. γ- and α-carotenes together made up about 10% of all the carotenoids detected and quantitated. The mean content of total carotenoids was 196.0 ± 83.2 μg/dl, with no statistically significant difference between levels for female and male subjects. The mean serum retinol level was 74.2 ± 23.0 μg/dl and none of the subjects in this study could be regarded to be vitamin A deficient.

Introduction

The measurement of the serum or plasma vitamin A level remains the most common biochemical means of assessing the status of nutriture of the vitamin. Plasma carotenoid concentration, on the other hand, is generally considered not to be a reliable indicator of vitamin A status because it reflects the level of immediate dietary intake. However, when considered with plasma retinol levels, carotenoid values can provide some insight into dietary patterns of individuals or populations (Arroyave *et al.*, 1982). Besides being of importance in relation to vitamin A status, the determination of serum retinol and carotenoids is now recognised to be of importance in studies of the relationship between these nutrients and cancer prevention and control (Tee, 1992).

A commonly used method for the analysis of serum retinol is based on the transient blue-coloured complex which retinol and its esters

form under anhydrous conditions with antimony trichloride-chloroform reagent (Carr-Price reaction). Other colour reagents used include trifluoroacetic and trichloroacetic acid. The major problem with the use of these methods is the evanescent nature of the blue colour obtained, posing considerable practical difficulty in taking readings in a colorimeter. For the determination of carotenoids in serum, the common method is to read the absorbance of the hexane extract at 450 nm. Results so obtained would, at best, be referred to as total carotenoid concentration.

In recent years, high-pressure liquid chromatography (HPLC) has become more widely used for the analysis of carotenoids and retinol, mainly because of the greater specificity of the technique (Tee & Lim, 1991a). A simple reversed-phase HPLC method has been developed by the authors in an effort to develop improved methodologies for the separation and quantitation of carotenoids and retinol in foods and biological specimens, especially blood serum. The method has been used successfully for the analysis of major carotenoids in Malaysian fruits and vegetables (Tee & Lim, 1991b) and the simultaneous determination of retinol and several carotenoids in foods of animal origin and processed foods (Tee & Lim, 1992). This paper reports the application of the same method for the simultaneous analysis of carotenoids and retinol in serum. The samples were also simultaneously determined using the direct spectrophotometric method for carotenoids and the Carr–Price method for retinol.

Materials and methods

Solvents and standards

Analytical-grade solvents were used for sample preparation, whereas solvents for high-pressure liquid chromatography were of HPLC grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45 μm regenerated cellulose membrane filter and degassed using an ultrasonic bath.

α- and β-carotenes, lycopene and retinol standards were purchased from Sigma Chemical Company. γ-Carotene, cryptoxanthin, and lutein were gifts from F. Hoffmann La-Roche, Switzerland. Stock solutions of the carotenoids were prepared in hexane (except that lutein was prepared in ethanol) and retinol in ethanol, in concentrations of 100 μg/ml and stored in amber bottles below −20°C. Working solutions of 1 μg/ml of the standards were prepared daily. For standards from Sigma, the appropriate absorptivities (extinction coefficients) given by the company were used to calculate the exact concentration of each of the compounds. For other carotenoids, absorptivities published in the literature (De Ritter, 1981) were used. Absorbance readings and absorption spectra of all standard solutions were monitored daily. The preparation of all standard solutions was carried out rapidly, in a room with subdued light and with all windows tinted with a light-protective film. All sample treatment and analytical procedures were also carried out in this room.

Samples and sample preparation

Serum retinol and carotenoid concentrations. Blood samples sent to the laboratory for determination of serum lipid profile were selected based on the following criteria: (1) samples were from apparently healthy subjects who requested routine medical examination; (2) samples were collected on the same day; and (3) sufficient volume was available. Blood samples meeting these criteria were allowed to clot and the sera separated by centrifugation. Aliquots of the sera from these samples were immediately taken and stored in the dark at −20°C. The samples were analysed within 1 week of collection.

Serum samples were processed for analysis using the following procedures. Into glass-stoppered 15 ml centrifuge tubes were added in sequence, 0.5 ml serum, 1.5 ml distilled water, and 2 ml 95% ethanol and the contents were mixed in a vortex. After the addition of 3 ml petroleum ether (b.p. 40–60°C), the tubes were stoppered and shaken vigorously for 2 min. The tubes were centrifuged for 5 min and 2 ml of the top petroleum ether layer (test solution) pipetted out for analysis by the HPLC method.

Comparative study of different analytical methods. Left-over human sera sent to the laboratory for determination of serum lipid profile were pooled for comparative studies of the HPLC method, the direct spectrophotometric method and the Carr–Price method for the analysis of carotenoids and retinol. Sera that were stored for more than 5 days in the refrigerator were not used.

Aliquots of the pooled sera were processed as follows for the comparative study. Into each of four glass-stoppered 15 ml centrifuge tubes were added 1 ml serum and 1 ml distilled water and processed as described above. After centrifuging the tubes, 2 ml of the top petroleum ether layer (test solution) were pipetted out from each tube and pooled for the required analyses. Two 2 ml aliquots of each of the test solutions were determined by the direct spectrophotometric and the Carr–Price methods for total carotenoid and retinol respectively. Another two 2 ml aliquots of each of the test solutions were analysed by the HPLC method for simultaneous determination of several carotenoids and retinol. In this way, 1 ml aliquots of pooled sera were determined in duplicate by each of the two sets of methods.

Extractant for retinol and carotenoids. Pooled sera were also used for a study of the effect of using the HPLC mobile phase instead of petroleum ether for extracting retinol and carotenoids from the sera. Retinol and carotenoid concentrations were simultaneously determined using the HPLC method.

High-pressure liquid chromatography method

HPLC conditions. A Gilson liquid chromatograph (Gilson Medical Electronics Inc., Middleton, USA) equipped with a 305 pump was used. A Gilson 116 UV detector set at 325 nm was connected in series with a Waters 440 fixed-wavelength detector (Millipore Corp., Milford, USA) at 436 nm to enable the simultaneous detection of retinol and carotenoids. A stainless steel 30 cm × 3.9 mm (i.d.) μBondapak C_{18} column (Millipore Corp., Milford, USA) was used for the chromatographic separation. This column was preceded by a precolumn module housing a disposable precolumn insert packed with the same material as that in the analytical column. The mobile phase used consisted of a ternary mixture of acetonitrile, methanol and ethyl acetate (88:10:2, v/v), delivered at a rate of 2 ml/min. The sample injection volume, dispensed with a Rheodyne 7125 injector (Rheodyne Inc., California, USA), was usually 50 μl. Data were analysed and stored using a Gilson 714 HPLC system controller software (Gilson Medical Electronics Inc., Middleton, USA), operating in a IBM-AT compatible microcomputer.

Chromatography of carotenoids and retinol. Two 2 ml aliquots of the test solution were evaporated to dryness in a 50°C waterbath with the aid of a stream of nitrogen. The residues were immediately re-dissolved in 2 ml mobile phase. After filtering through a 0.45 μm membrane filter, aliquots were injected into the HPLC for simultaneous detection and quantitation of retinol and carotenoids.

Peak areas of retinol, α- and β-carotenes, and lycopene were quantitated and their concentrations calculated using reference standards of these compounds similarly chromatographed. Total peak area obtained at 436 nm for each sample was used for calculating total carotenoid concentration in the sample.

Direct spectrophotometric and Carr–Price methods

The direct spectrophotometric and the Carr–Price methods were essentially as those given in the ICNND manual (ICNND, 1963). Two 2 ml aliquots of the test solution were placed in 10 × 75 mm cuvettes and the absorbances read immediately in a spectrophotometer at 450 nm against a petroleum ether blank. Total carotenoid concentrations were calculated from the absorbance reading using a calibration curve prepared using a β-carotene standard.

In the following determination of retinol for the two test solutions, each solution was analysed separately after completing one sample. The test solution in the cuvette was evaporated to dryness in a 50°C water bath with the aid of a stream of oxygen-free nitrogen. The residue was immediately re-dissolved in 0.1 ml chloroform, and one drop of acetic anhydride was added. The spectrophotometer was set to 620 nm and zeroed using a reagent blank consisting of 1 ml antimony trichloride reagent (20% antimony trichloride in chloroform), 0.1 ml chloroform and one drop of acetic anhydride. The test cuvette was placed in the spectrophotometer and 1 ml antimony trichloride reagent was delivered rapidly into the tube. The absorbance reading was taken at the pause point, about 3 s after the addition of the reagent.

Since carotenoids in the serum are also known to react with antimony trichloride, the absorbance reading at 620 nm obtained for the test sample was corrected as follows.

$Absorbance_{620}$ – ($Absorbance_{450}$ × correction factor)

The correction factor was determined as follows. β-Carotene standard solutions of various concentrations were read at 450 nm and 2 ml aliquots evaporated to dryness. After redissolving the residue in 0.1 ml of chloroform, one drop of acetic anhydride was added. The mixture was reacted with antimony trichloride reagent and the absorbance reading taken at 620 nm at the pause point. An absorbance reading @ 620 nm *vs* absorbance @ 450 nm was constructed and the slope of the curve taken as the correction factor.

The corrected absorbance reading at 620 nm was used for calculating retinol concentration in the test sample using a calibration curve prepared using retinol standard similarly reacted with the antimony trichloride reagent.

Statistical analyses

Student's *t* test was used for determining whether there were significant differences in mean retinol and carotenoid levels between male and female subjects. In the comparative study of the HPLC method and the direct spectrophotometric and the Carr–Price methods, statistical tests for significant differences between values for two sets of data were carried out using the Wilcoxon signed-rank test (two-tailed), the non-parametric method for not normally distributed observations. All statistical tests were carried out using the SPSS for Windows statistical package. For all tests carried out, a *P* value of <0.05 was taken as statistically significant.

Results and discussion

Simultaneous determination of carotenoids and retinol

The HPLC method described in this study was developed by the authors for the simultaneous determination of carotenoids and retinol in foods of animal origin (Tee & Lim, 1992). A similar approach was taken in this study, where a Waters 440 fixed-wavelength detector with a 436-nm filter was connected in series to a Gilson UV 116 detector set at 325 nm. The controller software was able to separately quantitate the areas of peaks detected by the two detectors. The software also enabled re-analyses of data, using the most appropriate integration

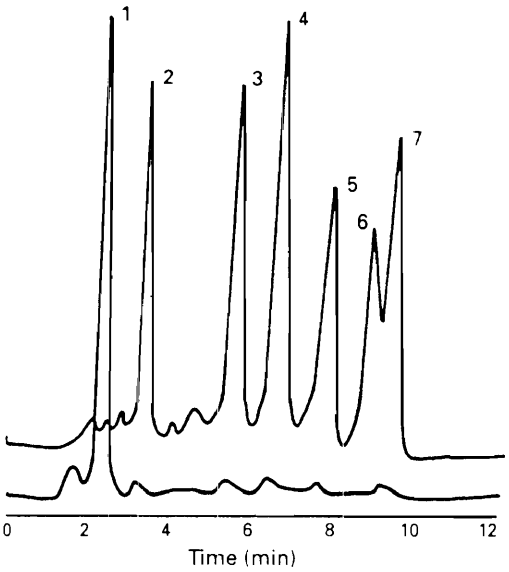

Figure 1. HPLC chromatogram of a mixture of retinol and carotenoid standards. Retinol and carotenoids were detected at 325 nm and 436 nm respectively, using two detectors connected in series. Other chromatography conditions are as given in the text. A volume of 50 µl of the standard mixture was injected. 1 = retinol; 2 = lutein; 3 = cryptoxanthin; 4 = lycopene; 5 = γ-carotene; 6 = α-carotene; 7 = β-carotene.

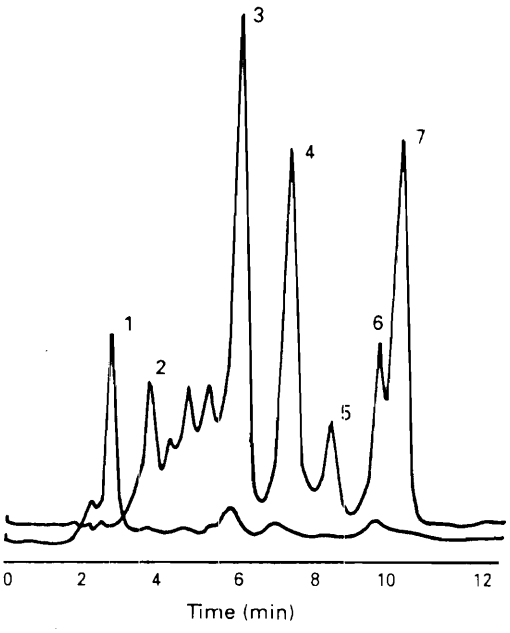

Figure 2. HPLC chromatogram of human serum. Chromatography conditions are as described for Figure 1. 0.5 ml serum was used for the analysis, and 300 µl sample extract was injected. 1 = retinol; 2 = lutein; 3 = cryptoxanthin; 4 = lycopene; 5 = γ-carotene; 6 = α-carotene; 7 = β-carotene.

Table 1. Age (years) of study subjects by sex and ethnic groups

	Combined	*Sex*		*Ethnic groups*		
		Male	*Female*	*Chinese*	*Malays*	*Indian*
Number	100	58	42	38	38	24
Mean	52.8	53.9	51.2	55.6	47.8	56.2
Median	54.0	53.0	54.0	55.5	45.0	56.0
SD	11.2	11.2	11.2	8.9	11.1	12.2
Minimum	17.0	27.0	17.0	28.0	17.0	27.0
Maximum	78.0	78.0	69.0	78.0	69.0	76.0

parameters for the peaks in each chromatogram. A chromatogram of retinol and carotenoid standards separated using the configurations described above is shown in Figure 1, while Figure 2 shows a chromatogram of a serum sample analysed using the method described.

Serum retinol and carotenoid concentrations of Malaysians

Age and sex distribution of subjects. The mean age of the 100 subjects studied was 52.8 years (Table 1). Sex ratio of male to female subjects was 1.4:1. Chinese and Malay subjects each constituted slightly over one third of the subjects studied, while less than one third were Indian subjects. Because of the manner of obtaining blood samples for study, there was little control over the selection of subjects for inclusion in the study in order to have a ratio of ethnic groups that was more reflective of the racial composition in the country. Thus ethnic differences in retinol and carotenoid concentrations were noted, but no significance tests were applied to the results obtained.

Serum retinol level. The mean serum retinol concentration of 100 apparently healthy Malaysians with no reported vitamin deficiencies was 74.2 μg/dl, and the median was 74.0 μg/dl (Table 2). The International Vitamin A Consultative Group (IVACG) (Arroyave *et al.*, 1982) has recommended that 20 μg/dl may be used as a cut-off for acceptable serum levels of vitamin A, without age differentiation. The mean serum retinol concentration of the sub-

Table 2. Serum retinol and carotenoid levels (μg/dl) of study subjects

	Retinol	*Lutein*	*Cryptoxanthin*	*Lycopene*	*γ-Carotene*	*α-Carotene*	*β-Carotene*	*Sum*	*Total*
All subjects (n = 100)									
Mean	74.2	35.4	35.5	22.7	6.5	9.1	29.2	138.3	196.0
Median	74.0	31.8	28.9	18.1	5.4	7.6	24.1	128.8	185.4
SD	23.0	13.5	20.5	13.9	3.8	6.7	21.3	61.8	83.2
Minimum	30.9	6.0	3.6	1.1	0.0	0.0	2.7	15.2	23.3
Maximum	167.1	71.2	105.7	68.3	19.5	39.6	116.9	335.8	448.4
Male (n = 58)									
Mean	77.5	37.0	36.0	21.6	6.4	8.4	25.5	134.8	194.6
Median	76.4	31.8	29.4	16.4	5.7	7.5	21.3	128.1	185.4
SD	22.8	14.0	20.6	13.0	3.7	6.4	16.4	55.7	77.0
Minimum	35.5	14.3	3.6	4.3	2.1	0.0	5.2	41.1	62.2
Maximum	167.1	70.8	98.6	68.3	19.5	39.6	80.9	325.3	443.1
Female (n = 42)									
Mean	69.6	33.2	34.7	24.2	6.6	10.1	34.3	143.2	198.0
Median	70.5	32.1	27.9	21.3	5.3	8.5	27.9	134.2	185.9
SD	22.6	12.6	20.6	15.1	3.8	7.1	26.0	69.8	92.1
Minimum	30.9	6.0	4.7	1.1	0.0	0.0	2.7	15.2	23.3
Maximum	135.6	71.2	105.7	56.8	18.4	31.7	116.9	335.8	448.4

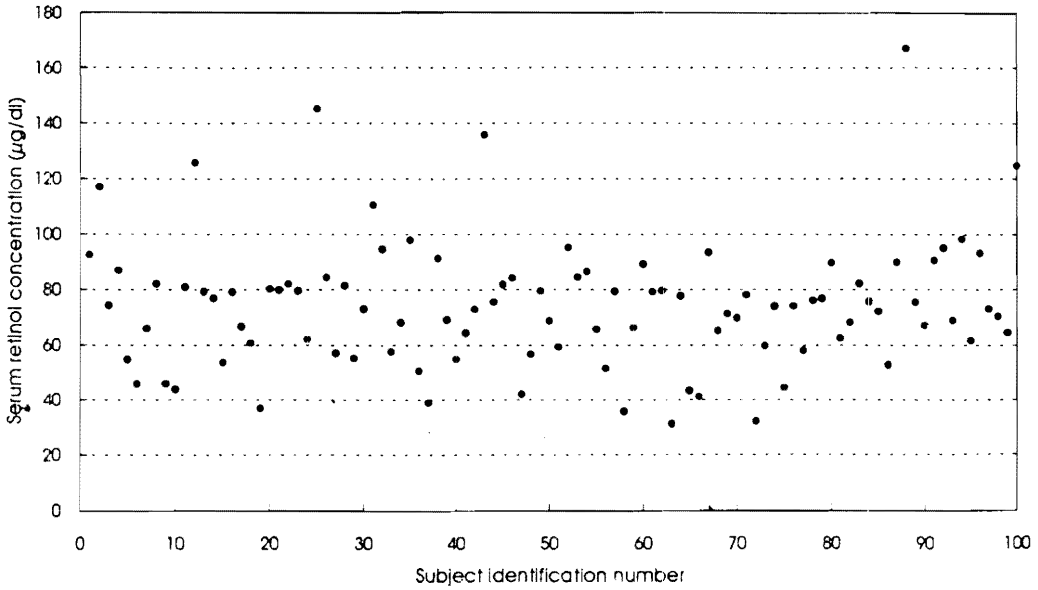

Figure 3. Distribution of serum retinol concentrations of study subjects (n = 100).

jects studied was well above this cut-off level. None of the subjects had a serum level below 20 µg/dl. Serum retinol concentrations of most of the subjects were clustered between 40 and 90 µg/dl (Figure 3).

There was no statistically significant difference between the mean retinol concentration of male subjects (77.5 µg/dl) and that of the female subjects (69.6 µg/dl) (Table 2). Mean retinol concentrations of the three ethnic groups were found to be similar (Table 3).

The mean serum retinol levels obtained for the present group of subjects (74.2 µg/dl) are higher than those reported earlier by this laboratory. For

Table 3. Serum retinol and carotenoid levels (µg/dl) of study subjects by ethnic groups

	Retinol	*Lutein*	*Cryptoxanthin*	*Lycopene*	*γ-Carotene*	*α-Carotene*	*β-Carotene*	*Sum*	*Total*
Chinese (n = 38)									
Mean	75.1	37.0	34.7	21.0	7.0	10.8	33.0	143.5	200.7
Median	77.9	35.0	31.1	16.9	5.9	9.0	26.1	136.8	189.1
SD	23.8	14.8	19.5	13.5	3.9	7.3	26.7	69.0	91.9
Minimum	36.7	6.0	3.6	1.1	0.0	0.7	2.7	15.2	23.3
Maximum	145.2	71.2	98.6	49.9	18.4	31.7	116.9	335.8	448.4
Malays (n = 38)									
Mean	73.9	36.1	38.9	27.1	6.9	10.0	28.4	147.4	210.0
Median	72.5	32.7	32.5	24.9	6.1	8.3	25.7	137.9	191.2
SD	24.4	12.5	22.4	14.8	4.2	6.6	16.1	60.7	83.3
Minimum	30.9	14.3	12.0	4.3	2.7	2.9	7.8	68.2	93.3
Maximum	167.1	70.5	105.7	68.3	19.5	39.6	80.9	325.3	443.1
Indian (n = 24)									
Mean	73.2	31.7	31.3	18.2	5.0	5.0	24.4	115.7	166.5
Median	74.2	28.0	26.3	14.7	5.1	5.0	17.0	107.3	156.6
SD	19.9	12.6	18.7	11.6	2.1	3.9	18.5	46.4	61.3
Minimum	35.5	14.1	10.8	6.1	1.6	0.0	9.3	48.6	68.2
Maximum	135.6	70.8	74.9	56.8	8.9	16.0	81.5	221.8	309.1

example in a series of studies of villages in Peninsular Malaysia conducted by this laboratory, mean serum retinol level of some 500 adults (18–45 years, sexes combined) was about 47 μg/dl (Chong *et al.*, 1984). The higher values can, in part, be explained by the differences in analytical methods. As will be discussed later in this report, the HPLC method described gives higher retinol values, by approximately 1.3 times, compared with the Carr-Price method used by Chong *et al.* (1984).

More importantly, the higher retinol levels could be because the subjects in the present series are relatively well-nourished urban executives. Because of the way the serum samples were obtained, it was not possible to determine the dietary intake of the subjects, including the possibility that the subjects could have been on vitamin supplements.

The US Interdepartmental Committee on Nutrition for National Defense (ICNND, 1963) had recommended 20–49 μg/dl as an 'acceptable' serum vitamin A level, and a level of >50 μg/dl as 'high'. Using these values, the serum vitamin A levels reported in this study, and even those of the rural villagers reported earlier, would be considered as 'high'. It would appear that these levels suggested by ICNND may need to be revised upwards.

Serum carotenoid concentrations. Table 2 tabulates the mean concentrations of the six major carotenoids found in the human sera studied. All the carotenoids were detected in all the serum samples, except α- and γ-carotenes. α-Carotene was not detected in four subjects, while γ-carotene was not detected in one subject. Concentrations of most of the carotenoids showed large variations.

The mean β-carotene concentration of the subjects studied was 29.2 μg/dl (Table 2), with most of the individual values clustered around 10–40 μg/dl (Figure 4). Blood concentrations of the carotenoid are generally accepted to be an unreliable indicator of vitamin A status because they reflect the level of immediate dietary intake. The guidelines for interpretation of serum carotene levels suggested by ICNND (1963) are not applicable to the β-carotene concentrations reported here. The values given in the guidelines should be more correctly termed as total carotenoids, as the colorometric method used was not able to quantitate the individual carotenoids.

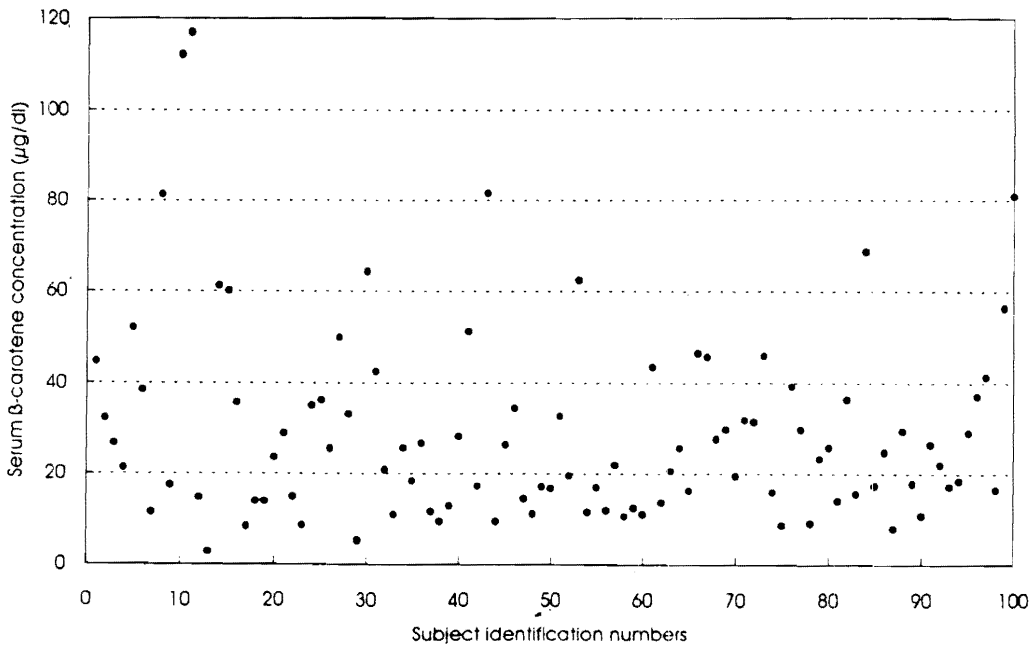

Figure 4. Distribution of serum β-carotene concentrations of study subjects (n = 100).

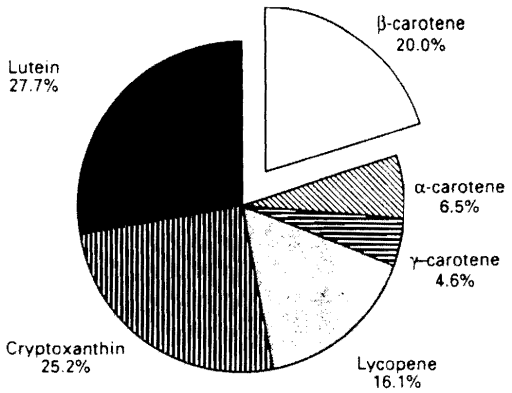

Figure 5. Proportions of major carotenoids in human sera (n = 100).

Mean concentrations of lycopene, γ- α- and β-carotenes of the female subjects were higher than those for the mean levels of the male subjects, but there was no statistically significant difference. The mean lutein and cryptoxanthin levels of the male subjects were higher than those of the female subjects, but again there was no statistically significant difference (Table 2).

Differences in the mean concentrations of the various carotenoids were observed for the serum samples from the Chinese, Indian and Malay subjects (Table 3). However, the mean levels were of the similar order of magnitude among the three ethnic groups.

The most abundant carotenoids in the serum samples studied were lutein and cryptoxanthin, each contributing to about one quarter of all the carotenoids quantitated (Figure 5). β-Carotene and lycopene were the next abundant carotenoids, each contributing to about 20% of all carotenoids, γ- and α-carotenes together made up about 10% of all the carotenoids detected and quantitated. Variations in these proportions were observed for the individual subjects (Table 4), but the order of magnitude was very much the same.

The mean total carotenoid concentrations of the subjects studied was close to 200 μg/dl (Table 2). None of the subjects was found to have a total carotenoid concentration of less than 40 μg/dl, the level considered to be 'low' in the ICNND guidelines. The female and male subjects were found to have very similar mean total carotenoid concentrations. A similar pattern of total carotenoid concentrations was observed for the three ethnic groups, although the mean level for the Indian subjects appeared lower than mean levels of the other two ethnic groups (Table 3).

Comparative study of different analytical methods

Retinol concentration. Table 5 shows that the mean retinol levels of 10 pooled sera studied by the HPLC method were higher than values given by the Carr–Price method. Ratios of results given by the HPLC method to those obtained by the Carr–Price method were <1.30 for most of the pools, with a mean ratio of 1.26 for all the 10 pools. When all the 33 pairs of results obtained by the two methods (each of the 10 pools were analysed in triplicate) were compared statistically using the Wilcoxon signed-rank test (two-tailed), the HPLC method was found to give significantly higher results.

The Carr–Price method, which has been widely used by investigators for the assessment of vitamin A status of individuals, has several disadvantages. The blue colour produced is

Table 4. Percentage of individual carotenoids

	Proportion (%) of individual carotenoids					
	Lutein	*Cryptoxanthin*	*Lycopene*	*γ-Carotene*	*α-Carotene*	*β-Carotene*
Mean	27.7	25.2	16.1	4.6	6.5	20.0
Median	26.8	23.4	15.0	4.5	6.1	19.3
SD	8.9	7.5	6.4	1.4	3.1	7.7
Minimum	10.5	8.8	5.5	0.0	0.0	8.9
Maximum	53.4	54.3	42.5	8.7	20.4	46.1

Number of subjects = 100.

Table 5. Retinol concentration (μg per dl) of human pooled sera determined by the Carr-Price and the HPLC methods

	Carr–Price method		*HPLC method*		*Ratio of HPLC Carr–Price*	
	Mean[a]	*SD*	*Mean*	*SD*	*Mean*	*SD*
1	40.9	0.24	51.3	3.05	1.25	0.08
2	56.5	2.65	67.2	4.74	1.19	0.05
3	55.4	1.87	79.5	1.96	1.44	0.06
4	49.9	0.79	61.5	2.19	1.23	0.06
5	56.0	2.58	65.8	2.31	1.18	0.05
6	54.8	0.78	67.9	1.36	1.24	0.04
7	54.2	1.84	64.7	3.23	1.19	0.08
8	54.9	3.55	66.9	9.63	1.22	0.13
9	53.3	0.24	69.1	3.31	1.30	0.07
10	51.1	0.29	68.3	1.61	1.34	0.04

[a] Each value is the mean of three analyses performed on different days.

transient and therefore demands a great deal of skill on the part of the laboratory worker. Corrections have to be made for the presence of carotenoids which also react with antimony trichloride. This colour reagent is also very sensitive to the presence of moisture which renders the solution turbid and unsuitable for reading. The reagent also forms a film on the cuvette which cannot be removed.

Total carotenoid concentration. The mean total carotenoid concentrations of the 10 pooled sera studied by the HPLC method were higher than the mean values given by the direct spectrophotometric method (Table 6). Ratios of mean results given by the HPLC method to the direct spectrophotometric method ranged from 1.18 to 1.88, with a mean of 1.38 for all the 10 pools. Statistical analysis of all the 33 pairs of results showed that the HPLC method gave significantly higher results.

The direct spectrophotometric method has often been used for assessing the carotene status of individuals. Sometimes, the results have been erroneously reported as β-carotene. In the ICNND manual (ICNND, 1963), results

Table 6. Total carotenoid concentration (μg per dl) of human pooled sera determined by the direct spectrophotometric and HPLC methods

	Direct spectrophotometric method		*HPLC method*		*Ratio of HPLC to direct spectrophotometric method*	
	Mean[a]	*SD*	*Mean*	*SD*	*Mean*	*SD*
1	118.0	3.84	142.0	11.90	1.20	0.11
2	143.0	2.06	176.0	11.60	1.24	0.10
3	176.0	2.33	225.0	15.40	1.28	0.09
4	126.0	4.32	183.0	7.89	1.45	0.09
5	132.0	5.80	181.0	12.70	1.38	0.14
6	160.0	6.75	301.0	28.50	1.88	0.10
7	160.0	4.03	209.0	17.20	1.30	0.07
8	136.0	3.52	206.0	33.80	1.51	0.22
9	150.0	2.31	177.0	9.44	1.18	0.05
10	168.0	4.35	239.0	22.90	1.43	0.10

[a] Each value is the mean of three analyses performed on different days.

Table 7. Retinol and carotenoid concentrations obtained using two extractants

	Retinol		*Total carotenoids*		*β-Carotene*		*α-Carotene*		*Lycopene*	
	PE	*MP*	*PE*	*MP*	*PE*	*MP*	*PE*	*MP*	*PE*	*MP*
Pool 1	64.4	63.9	171.0	137.0	18.8	7.0	5.9	2.6	12.9	5.8
Pool 2	70.2	73.2	255.0	170.0	52.1	13.5	13.5	2.8	22.9	7.4

PE = petroleum ether.
MP = HPLC mobile phase.

obtained by direct spectrophotometry were referred to as 'carotene'. Total carotenoid concentrations determined by the direct spectrophotometric and the HPLC methods as described may only be taken as approximations – firstly because absorbance reading at 450 nm for the former method and detection at 436 nm for the latter are only approximations since carotenoids have rather varied absorption maxima; and secondly because calculation based on a β-carotene standard is a further approximation since carotenoids have varying absorptivities. Nevertheless, an estimation of total carotenoid concentration would be useful as it would then be possible to estimate proportions of various individual carotenoids. For this purpose, the direct spectrophotometric method is easy to carry out.

Mobile phase as extractant

If the HPLC mobile phase could be used as an extractant of the retinol and carotenoids from the serum samples, the solvent would not have to be evaporated dry and could therefore reduce the loss of these compounds through heating. Results obtained on two pooled sera using the mobile phase as an extractant showed that retinol concentrations obtained were very close to those obtained using petroleum ether (Table 7). However, total carotenoid concentrations as well as levels of the three major carotenoids were much lower when the mobile phase was used as the extractant.

Conclusions

The HPLC method developed in this study is relatively simple, and is applicable to the analysis of carotenoids in fruits and vegetables, as well as to the simultaneous determination of carotenoids and retinol in foods of animal origin and human sera. A volume of 0.5 ml of serum was sufficient for satisfactory separation and quantitation of carotenoids and retinol. If only retinol concentration is required, the volume of blood could even be halved. The absorbance reading of the petroleum ether extract could be obtained for an estimation of the total carotenoid concentration, from which the proportions of individual major carotenoids could be estimated.

The HPLC method has advantages over the direct spectrophotometric method for the determination of carotenoids since the former is able to separate and quantitate the various carotenoids present in the serum. It is also preferred over the Carr–Price method for the determination of retinol since it is more specific and overcomes the various problems associated with the use of antimony trichloride or similar reagents. Furthermore, the HPLC method is able to simultaneously quantitate carotenoids and retinol in a single chromatographic run if two detectors are connected in series.

Data on the carotenoid profile of serum of different population groups are still lacking. In view of the increasing interest in the protective role of β-carotene and other carotenoids in some forms of cancer, the present HPLC procedure should facilitate more refined epidemiological and experimental studies into such a relationship.

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