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Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects

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Carotenoid and vitamin C intakes, assessed by FFQ, have been positively associated with plasma concentrations in different populations. However, the influence of BMI on these associations has not been explored in detail. We explored in a cross-sectional study the relation between dietary carotenoid and vitamin C intakes, using a 135-item FFQ, with their plasma concentrations by BMI categories in 252 men and 293 women, 65 years and older. For men and women combined, significant ($P<0{\cdot}05$) Pearson correlations were observed between energy-adjusted dietary intakes and plasma concentrations (carotenoids adjusted for cholesterol) for: α-carotene 0·21, β-carotene 0·19, lycopene 0·18, β-cryptoxanthin 0·20 and vitamin C 0·36. Multiple linear regression analyses showed that the intake of carotenoids and vitamin C were significant predictors of their respective plasma concentration ($P<0{\cdot}01$), and that BMI was inversely associated with plasma concentration of carotenoids ($P\leq0{\cdot}01$) but not with plasma vitamin C. In addition, we observed significant interactions between BMI and the intakes of α-carotene and lutein + zeaxanthin, and to a lower extent β-carotene, suggesting that these intakes in subjects with high BMI were not good predictors of their plasma concentration. The present data suggest that plasma carotenoids and vitamin C may be good markers of dietary intake in elderly subjects, but not so for α-carotene, β-carotene and lutein + zeaxanthin in obese subjects.

Carotenoids: Vitamin C: Food frequency questionnaire: Plasma concentrations

Epidemiologic studies have consistently shown protective effects of fruit and vegetable consumption against cancer, CVD and other chronic diseases (Glade, 1999; van't Veer *et al.* 2000; Hung *et al.* 2004). However, this protective effect seems to be less evident for some chronic diseases such as age-related maculopathy, particularly when usual dietary intake of antioxidant nutrients such as lutein, zeaxanthin, vitamin C and vitamin E, mostly contained in fruits and vegetables, are considered (Cooper *et al.* 1999; Smith *et al.* 1999; Mares-Perlman *et al.* 2001; Cho *et al.* 2004; Stanner *et al.* 2004; van Leeuwen *et al.* 2005). This discrepancy might be due in part to the approach used to assess the long-term dietary intake of antioxidant nutrients such as carotenoids or vitamin C. Epidemiologic studies examining the role of these antioxidant vitamins can rely upon either questionnaires or biochemical markers in plasma or tissues to represent the long-term dietary intake, but their interpretations may differ.

Questionnaire-based intakes of carotenoids and vitamin C may have advantages in terms of better capturing long-term exposure and identifying associations with chronic disease risk, although they may also be subject to biased reporting or faulty recall particularly when applied to special groups such as the elderly (Jacques *et al.* 1993). Carotenoids and vitamin C in plasma may be influenced not only by dietary intake and external factors such as the food matrix and the food preparation that ultimately can affect bioavailability (Hunter, 1998; Mayne, 2003), but also by host factors such as gender, smoking status and BMI. The effect of smoking and gender on diet–plasma correlations of vitamins has been reported (Brady *et al.* 1996; Ritenbaugh *et al.* 1996; Michaud *et al.* 1998; Tucker *et al.* 1999), but to our knowledge no study has examined the effect of stratifying the diet–plasma correlations by groups of BMI.

Cross-sectional studies have demonstrated that carotenoid concentrations in plasma and adipose tissue were inversely related to BMI (Brady *et al.* 1996; Scott *et al.* 1996; Vogel *et al.* 1997; Neuhouser *et al.* 2001; Wallstrom *et al.* 2001; Galan *et al.* 2005). In a longitudinal study it was shown that BMI predicted the evolution of serum carotenoids during a 7-year follow-up among young non-smoking adults with the exception of lycopene (Andersen *et al.* 2006). Other cross-sectional studies have also failed to observe associations between BMI and lycopene (Brady *et al.* 1996; Neuhouser *et al.* 2001; van Kappel *et al.* 2001) as well as with β-cryptoxanthin and lutein + zeaxanthin (Tucker *et al.* 1999; Al Delaimy *et al.* 2005).

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In the present paper, we report the association between major carotenoid and vitamin C intakes derived from a FFQ and their serum concentrations in a cross-sectional study with a sample of the elderly population aged 65 and over participating in the EUREYE study in Spain. We explored whether this association was influenced by age, sex, smoking status and, particularly, BMI. To our knowledge there is no published study which grouped the study population by BMI category in order to investigate whether correlations between plasma concentrations of these nutrients and their respective dietary intake differed. The present results may provide an explanation for the lack of consistency in the association between antioxidant intake and risk of some chronic diseases.

Subjects and methods

Sample design and study population

Participants in this cross-sectional study were 600 apparently healthy subjects, 274 men and 326 women, recruited in the province of Alicante, Spain, for the EUREYE study, a multi-centre, population-based, cross-sectional study carried out in seven European countries. All the interviews and clinical examinations were carried out between February 2000 and November 2001. The main objectives of the EUREYE study were to identify lifestyle and environmental determinants of age-related maculopathy and macular degeneration in the European setting, with a particular focus on solar radiation and diet. A detailed description of the study design has been reported (Augood *et al.* 2004, 2006). The random sample was drawn by the statistics officers at the registry, and was based on the sampling frame consisting of all persons aged 65 or over who were on the National Office for Statistics Census at the time the sample was requested. Ethical approval for the study was given by the Local Ethical Committee of the Hospital de San Juan and the University Miguel Hernandez, Alicante, Spain. Written informed consent was obtained from all subjects. Fifty-five subjects were excluded from the analysis because of missing data since they were unwilling to provide a blood sample. Therefore, 545 subjects (252 men and 293 women) were included in the final analysis.

Data collection

Study participants were interviewed by trained fieldworkers using structured questionnaires for socio-demographic details, current and past smoking habits, and dietary habits (FFQ). At the clinical examination, fieldworkers in all centres followed the same standard protocol, with some additional questions and anthropometric measurements only recorded in Spain. Height was measured to the nearest 0·1 cm with subjects standing without shoes and with their backs to the stadiometer. Body weight was measured in light clothes to the nearest 0·1 kg on a digital scale (Tefal® Top-Line, Barcelona, Spain) which was placed on a firm flat surface. BMI [weight (in kg)/height^2 (in m)] was calculated from weight and height measurements.

Dietary assessment

A semi-quantitative FFQ of 135 food items was used to assess usual dietary intake. The FFQ, an expanded modification of the Harvard questionnaire (Willett *et al.* 1985; Willett, 1998), contained a core list of food items common to all participating countries in the EUREYE study. Additional food items, and the local variety of some food items, were added to the core list in every country in order to capture the major sources of a considerable variety of nutrients, including specific carotenoids. In the Spanish centre, the added food items were mostly selected from a previous FFQ also based on the Harvard questionnaire that was adapted for use among elderly subjects with eye disease (Valero *et al.* 2002). This FFQ was previously validated using diet records in the same region from which the population of the present investigation was drawn. The validity and reproducibility correlation coefficients (adjusted for energy intake) ranged from 0·38 for reproducibility of carotenoids to 0·44 for validity of vitamin C (Vioque & Gonzalez, 1991; Vioque, 1995), a similar range to other established diet questionnaires (Willett, 1998).

Participants in the present study were asked how often, on average, they had consumed each type item over the past year. Serving sizes were specified for each food item in the FFQ. The questionnaire had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. Additionally, we modified the questionnaire to ask whether study participants followed special diets, and we included questions on the use of vitamin supplements (by type and frequency). Nutrient values were primarily obtained from food composition tables from the US Department of Agriculture (2005) and other published sources (O'Neill *et al.* 2001). Carotenoid intake, including α-carotene, β-carotene, lycopene, β-cryptoxanthin and lutein + zeaxanthin, was derived using the USDA–National Cancer Institute carotenoid database developed by Chug-Ahuja *et al.* (1993) and Mangels *et al.* (1993) and the European database to complete information on some specific foods and missing values (O'Neill *et al.* 2001). We calculated nutrient intakes by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the FFQ and by addition across all foods to obtain a total nutrient intake for each individual.

Blood collection and analysis

Peripheral venous blood samples were collected from each participant and analysed in a central laboratory (Queen's University, Belfast); 85 % of participants were in a non-fasting state. All participants were instructed by the interviewers not to consume fruits, vegetables or juices for breakfast on the day of the blood test. A thorough protocol was designed to collect, transport and measure the blood samples for vitamin C and carotenoids. Blood samples were separated by centrifugation and stored at −80°C. The blood samples for vitamin C determination were collected at the clinical examination under subdued light, were wrapped in tin foil, stabilized with metaphosphoric acid and placed in insulated dry containers at 4°C to exclude light and, therefore, avoid vitamin C degradation. Blood samples packed in dry ice were shipped to the Central Laboratory (Queen's University, Belfast) on a monthly basis, using dedicated couriers. Plasma cholesterol was measured to adjust carotenoid concentrations. Serum carotenoids were measured by HPLC with diode array detection as described by Craft (1992). Lutein + zeaxanthin plasma concentrations

were combined because information for these nutrients is combined in the main food composition tables. Plasma vitamin C was measured using an ascorbate oxidase-based assay as described by Vuillemier & Keck (1989). The inter-assay CV were $<10{\cdot}0\,\%$ and intra-assay CV $<5{\cdot}0\,\%$ for all species. Carotenoid and vitamin C assays were standardized against the appropriate National Institute of Standards and Technology standard reference materials.

Statistical analysis

Statistical analysis was performed with the STATA software package version SE/8.2 (StataCorp LP, College Station, TX, USA).

Carotenoid and vitamin C intakes were adjusted for total energy intake by calculating the residuals from a linear regression with the log *e* of the nutrient modelled as the dependent variable and the log *e* of total energy intake as the independent variable (Willett, 1998). Log *e* plasma carotenoid concentrations, but not log *e* plasma vitamin C, were regressed on plasma cholesterol because most carotenoids are transported in plasma lipoproteins, which may contribute to extraneous variation in the plasma carotenoids (Willett, 1998). Concerning carotenoids, the log *e* energy-adjusted intakes and the log *e* cholesterol-adjusted plasma concentrations were used in all the analyses except the multiple regression analyses. Concerning vitamin C, the log *e* energy adjusted intake and the log *e* plasma concentration were used in all analyses except multiple regression analyses. In the multiple regression analyses, the log *e* transformation of nutrient intakes and the log *e* of their plasma concentrations were used for carotenoids and vitamin C. Descriptive results were expressed as percentages or means and standard deviations using untransformed data.

We used unpaired Student's *t* tests, one-factor ANOVA and the Bonferroni *post hoc* test for means comparison of continuous variables between men and women, and by categories of BMI according to the cut-off points of the WHO to define normal weight ($\text{BMI} < 25\,\text{kg/m}^2$), overweight ($\text{BMI}\ 25\text{–}30\,\text{kg/m}^2$) and obesity ($\text{BMI} \geq 30\,\text{kg/m}^2$). χ^2 tests were used for categorical variables. We calculated the Pearson correlation coefficients between dietary and plasma measures for each individual nutrient. We also estimated partial correlation coefficients for the total group and men and women adjusting for sex (only in case of the total group), age, smoking status, BMI, alcohol intake, supplement intake and fasting state. In addition, we performed partial analysis for each category of BMI adjusting for sex, age, smoking status, alcohol intake, supplement intake and fasting state. Since the results by BMI categories did not differ substantially between men and women, only results for the total group are presented.

We performed multivariate linear regression analyses to explore predictors of the plasma carotenoids and vitamin C concentrations. In addition to the respective intake of carotenoids or vitamin C, all models included as independent variables age (continuous variable), sex (two categories: men, women), BMI (continuous variable, log *e* transformed), alcohol intake (three categories: 0, 0·1–11·9, >12 g/d alcohol), supplement intake (two categories: yes, no), smoking habit (two categories: ex/non-smoker, current smoker), fasting state (two categories: fasting state, non-fasting state), plasma cholesterol (except model of vitamin C; continuous variable, log *e* transformed) and daily energy intake (continuous variable, log *e* transformed). Since the results by BMI categories did not differ substantially between men and women, we only present results for the total group. After models with main effects were fitted for specific carotenoids and vitamin C, we explored the significance of adding interaction terms between BMI and every nutrient intake. All tests are two-sided and a 5 % level of significance was used.

Results

The main characteristics of the subjects are presented for men and women in Table 1. The mean age of the study population was 73·5 years and women were older than men. Height and weight were lower in women than men, although BMI was higher. The average BMI of the study population was $29{\cdot}3\,\text{kg/m}^2$; overweight ($\text{BMI}\ 25\text{–}30\,\text{kg/m}^2$) and obesity ($\text{BMI} \geq 30\,\text{kg/m}^2$) were reported in 47·9 and 36·5 % of the study population, respectively. The prevalence of overweight was higher in men than in women but more women were classified as obese. Current smoking was reported by 14·7 % of the study population, and was reported in more men than women. More than 92 % of women had never smoked. The mean serum cholesterol concentration of the subjects was 5·55 mmol/l, and was significantly higher in women than in men. The use of dietary supplements containing vitamins was reported by 6·2 % of the participants (*n* 34).

With regard to dietary intake, women reported lower energy and cholesterol intake than men. Protein and carbohydrate intakes (% of energy) were significantly lower in men than women. Fat intake (% of energy) was not different between men and women (Table 1).

Intake of major carotenoids and vitamin C, and their plasma concentrations, are presented in Table 2. In general, women had significantly higher intakes of α-carotene, β-carotene and β-cryptoxanthin. Lycopene, lutein + zeaxanthin and vitamin C intake were also higher in women but differences were not significant. Accordingly, women showed significantly higher plasma concentrations of α-carotene, β-carotene and β-cryptoxanthin, and also of lycopene and vitamin C, compared to men.

Correlations between cholesterol-adjusted plasma carotenoid concentrations and their respective energy-adjusted dietary intakes as well as the correlations between plasma vitamin C and energy-adjusted dietary vitamin C intake, are shown in Table 3. In general, positive and significant coefficient correlations were observed for all nutrients in the total group. Higher correlation coefficients for α-carotene, lycopene, β-cryptoxanthin and vitamin C were observed in men than in women. In women, the correlation coefficient of β-carotene was higher than in men. The highest correlation coefficients were seen for vitamin C, particularly in men. Adjusting by sex (in case of the total group), age, smoking status, alcohol intake, supplement intake, fasting state and BMI (partial correlations) did not change the correlation coefficients of carotenoids, but decreased the coefficients of vitamin C in the total group and in men (Table 3).

The daily intake and plasma concentration of carotenoids and vitamin C by BMI categories of men and women combined are shown in Table 4. No statistically significant

Table 1. General characteristics and dietary intake profile of Spanish elderly subjects by sex (Mean values and standard deviations)

	Men (*n* 252)		Women (*n* 293)		
	Mean	SD	Mean	SD	*P* value
Age (years)	72·8	5·5	74·1	6·4	0·018
Height (cm)	163·5	6·6	150·2	5·6	<0·0001
Weight (kg)	76·9	12·2	67·2	12·0	<0·0001
BMI (kg/m^2)	28·7	3·9	29·7	4·9	0·008
Categories of BMI (%)					
< 25 kg/m^2	15·5		15·7		
25–30 kg/m^2	55·2		41·6		
≥ 30 kg/m^2	29·4		42·7		0·003
Smoking status (%)					
Never smokers	23·0		92·2		
Former smokers	49·2		4·4		
Current smokers	27·8		3·4		<0·0001
Cholesterol (mmol/l)	5·24	1·0	5·81	1·0	<0·0001
Non-fasting state (%)	86·1		83·6		0·410
Supplement intake (%)	6·3		6·1		0·921
Energy intake (kJ/d)	7164	1855	6531	1654	<0·0001
Total fat (% of energy)	38·1	5·3	38·7	5·4	0·206
Protein (% of energy)	18·9	2·6	20·3	3·0	<0·0001
Carbohydrates (% of energy)	43·0	6·7	44·7	6·3	0·002
Cholesterol intake (mg/d)	249·6	83·1	222·1	69·7	<0·0001
Alcohol intake (g/d)	11·7	14·6	2·8	4·6	<0·0001

differences were observed for the nutrient intakes except for the lutein + zeaxanthin intake which was lower in subjects with BMI < 25 compared to BMI 25–30 and BMI ≥ 30 (Bonferroni *post hoc* test, $P=0{\cdot}012$ and $P=0{\cdot}019$, respectively). Daily intake of fruit and vegetables did not differ by BMI categories. With regard to plasma concentrations, lower mean carotenoid concentrations were observed in general with increasing BMI category. The use of the Bonferroni *post hoc* test showed that plasma concentrations of α-carotene ($P=0{\cdot}001$), β-carotene ($P=0{\cdot}010$) and lycopene ($P=0{\cdot}006$) were significantly lower in subjects with BMI ≥ 30 than BMI < 25. The plasma concentration of lutein + zeaxanthin in subjects with BMI ≥ 30 was lower than that observed in subjects with BMI < 30 but differences did not reach statistical significance. Plasma vitamin C and β-cryptoxanthin concentrations did not vary significantly by BMI category.

The Pearson and partial correlations between cholesterol-adjusted plasma concentrations (for carotenoids) and their respective energy-adjusted dietary intakes by BMI categories are shown in Table 5. Regarding carotenoids, the highest Pearson correlations were observed in general among subjects with BMI < 25, except for lutein + zeaxanthin which showed higher correlations among participants with BMI 25–30. The highest correlations were observed for α-carotene and

Table 2. Dietary intake and plasma concentrations of carotenoids and vitamin C among Spanish elderly subjects by sex† (Mean values and standard deviations)

	Men (*n* 252)		Women (*n* 293)	
	Mean	SD	Mean	SD
Dietary intake				
α-Carotene (μg/d)	720·3	558·7	832·2**	504·2
β-Carotene (μg/d)	4000·1	2076·4	4358·2*	2134·3
Lutein + zeaxanthin (μg/d)	4283·0	2387·3	4600·1	3063·3
Lycopene (μg/d)	4009·4	2396·3	4042·8	2634·7
β-Cryptoxanthin (μg/d)	276·1	184·9	313·6*	231·8
Vitamin C (mg/d)	125·4	64·1	136·2	69·8
Plasma concentrations (μmol/l)				
α-Carotene	0·061	0·07	0·096****	0·14
β-Carotene	0·196	0·23	0·304****	0·34
Lutein + zeaxanthin	0·139	0·13	0·153	0·16
Lycopene	0·474	0·56	0·703**	0·86
β-Cryptoxanthin	0·107	0·13	0·136**	0·15
Vitamin C	38·3	19·5	51·0****	18·4

Mean values were significantly different from those of the men: *$P<0{\cdot}05$; **$P<0{\cdot}01$; ****$P<0{\cdot}0001$. All *P* values were computed based on total energy-adjusted log *e* values for intake of carotenoids and vitamin C (Willett, 1998), plasma cholesterol-adjusted log *e* values for plasma carotenoids (Willett, 1998) and log *e*-transformed data for plasma vitamin C.

† Descriptive means and standard deviations are presented using untransformed data.

Table 3. Correlations between plasma nutrient (carotenoid and vitamin C) concentrations and dietary intakes in Spanish elderly subjects† (Correlation coefficients)

	Pearson correlation			Partial correlation‡		
Nutrient	All cases (*n* 545)	Men (*n* 252)	Women (*n* 293)	All cases (*n* 545)	Men (*n* 252)	Women (*n* 293)
α-Carotene	0·21****	0·21**	0·17**	0·19****	0·18**	0·18**
β-Carotene	0·19****	0·14*	0·20***	0·17****	0·11*	0·21****
Lutein + zeaxanthin	0·10*	0·07	0·13*	0·12**	0·07	0·15*
Lycopene	0·18****	0·21***	0·14*	0·17****	0·20**	0·15*
β-Cryptoxanthin	0·20****	0·23***	0·16**	0·19****	0·21**	0·18*
Vitamin C	0·36****	0·36****	0·27****	0·29****	0·31****	0·26****

Mean values were significantly different from those of the men: *$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

† All analyses were performed with total energy-adjusted log *e* values for intake of carotenoids and vitamin C (Willett, 1998), plasma cholesterol-adjusted log *e* values for plasma carotenoids (Willett, 1998) and log *e*-transformed data for plasma vitamin C.

‡ Partial correlation coefficients adjusted for sex (in analyses for total group), age, smoking status, BMI, alcohol intake, supplement intake and fasting state.

β-carotene, and to a lesser extent, β-cryptoxanthin and lycopene, among subjects with BMI < 25. The lowest correlations were seen for lutein + zeaxanthin, with no clear pattern by BMI category. Adjustment for sex, age, smoking, alcohol intake, supplement intake and fasting state (partial correlations) led to a slight decrease in most correlations. Vitamin C reached the highest Pearson correlation coefficients in subjects with BMI < 25 but did not differ between subjects with BMI 25–30 and BMI ≥ 30. Performing the partial correlation analysis decreased the correlation coefficients.

The results from the multivariate regression analysis evidenced that the intakes of each carotenoid as well as of vitamin C were significant predictors of their plasma concentrations (Table 6). However, these main effect models that included age, BMI, alcohol intake, supplement intake, smoking habit, fasting state, plasma cholesterol (except vitamin C), daily energy intake, and the respective nutrient intake, explained, in general, a low percentage of the variability of plasma concentrations: plasma α-carotene 14·7 %; β-carotene 12·3 %; lutein + zeaxanthin 6·4 %; lycopene 8·8 %; β-cryptoxanthin 6·9 %; vitamin C 20·8 %. In these models, alcohol intake was negatively associated with α-carotene and β-carotene concentrations ($P<0·05$), and smoking inversely associated with vitamin C concentration ($P<0·05$). The BMI was inversely associated with α-carotene, β-carotene, lutein + zeaxanthin, lycopene ($P<0·05$), β-cryptoxanthin ($P=0·060$), but not with plasma vitamin C.

When we explored interactions between BMI and every nutrient intake (Table 6, model 2), significant interactions were found for α-carotene ($P=0·003$), lutein + zeaxanthin ($P=0·034$) and, to a lesser extent, for β-carotene ($P=0·071$), but not for lycopene ($P=0·458$), β-cryptoxanthin ($P=0·986$) or vitamin C ($P=0·178$). The addition of these significant interaction terms to the main effect models caused small changes in the prediction of the models as denoted by the model R^2 (Table 6).

Table 4. Dietary intake and plasma concentrations of carotenoids and vitamin C among Spanish elderly subjects by BMI categories (kg/m^2)†

(Mean values and standard deviations)

	BMI < 25 (*n* 85)		BMI 25–30 (*n* 261)		BMI ≥ 30 (*n* 199)		
	Mean	SD	Mean	SD	Mean	SD	*P* value‡
Dietary intake							
Energy intake (kJ/d)	7134	1800	6900	1880	6586	1599	0·041
α-Carotene (μg/d)	765·9	560·6	794·3	581·0	768·4	449·4	0·515
β-Carotene (μg/d)	3931·4	2076·1	4297·0	2404·8	4167·3	1674·7	0·107
Lutein + zeaxanthin (μg/d)	3971·9	2729·3	4565·6	3125·7	4512·0	2241·2	0·010
Lycopene (μg/d)	4209·3	2916·8	4227·6	2751·1	3687·0	1945·7	0·154
β-Cryptoxanthin (μg/d)	269·2	149·8	311·0	246·2	288·6	183·8	0·433
Vitamin C (mg/d)	124·1	59·8	139·1	77·8	123·9	53·5	0·156
Fruit intake (g/d)	229·1	96·2	257·2	117·8	253·5	92·6	0·100
Vegetable intake (g/d)	211·6	115·2	229·8	160·9	201·7	113·9	0·093
Plasma concentration (μmol/l)							
α-Carotene	0·140	0·23	0·077	0·08	0·059	0·05	0·001
β-Carotene	0·393	0·53	0·249	0·25	0·201	0·18	0·013
Lutein + zeaxanthin	0·163	0·19	0·157	0·16	0·126	0·11	0·132
Lycopene	0·926	0·27	0·608	0·69	0·444	0·42	0·005
β-Cryptoxanthin	0·130	0·18	0·135	0·16	0·103	0·11	0·033
Vitamin C	42·5	21·9	45·8	19·8	45·4	19·2	0·103

† Descriptive means and standard deviation are presented using untransformed data.

‡ *P* value from one-factor ANOVA test. For results of Bonferroni *post hoc* test see Results section. All *P* values were computed based on total energy-adjusted log *e* values for intakes of carotenoids, vitamin C, fruits and vegetables (Willett, 1998), plasma cholesterol-adjusted log *e* values for plasma carotenoids (Willett, 1998) and log *e*-transformed data for plasma vitamin C and energy intake.

Table 5. Correlations between plasma nutrient (carotenoid and vitamin C) concentrations and dietary intakes in Spanish elderly subjects by BMI category†

(Correlation coefficients)

	Pearson correlations			Partial correlations‡		
Nutrient	BMI < 25 (*n* 85)	BMI 25–30 (*n* 261)	BMI ≥ 30 (*n* 199)	BMI < 25 (*n* 85)	BMI 25–30 (*n* 261)	BMI ≥ 30 (*n* 199)
α-Carotene	0·41***	0·24***	0·10	0·34**	0·21**	0·11
β-Carotene	0·35*	0·15*	0·20**	0·31**	0·11	0·19**
Lutein + Zeaxanthin	0·10	0·20**	−0·04	0·11	0·20**	−0·02
Lycopene	0·23*	0·15*	0·21**	0·23*	0·15*	0·20**
β-Cryptoxanthin	0·26*	0·20**	0·17*	0·20*	0·19**	0·18*
Vitamin C	0·41***	0·35****	0·33****	0·38**	0·26****	0·26****

*$P<0·05$; **$P<0·01$; ***$P<0·001$; ****$P<0·0001$.

† All analyses were performed with total energy-adjusted log *e* values for intake of carotenoids and vitamin C (Willett, 1998), plasma cholesterol-adjusted log *e* values for plasma carotenoids (Willett, 1998) and log *e*-transformed data for plasma vitamin C.

‡ Partial correlation coefficients adjusted for sex (in analyses for total group), age, smoking status, BMI, alcohol intake, supplement intake and fasting state.

Discussion

The main result of the present cross-sectional study, which was carried out in an elderly population with a high prevalence of overweight and obesity, was that the nutrient intakes of carotenoids and vitamin C were in general significant predictors of their respective plasma concentrations. However, for α-carotene, β-carotene and lutein + zeaxanthin intakes we found significant interactions with BMI which might indicate that the intakes of these three carotenoids were not predictive or much less predictive of their plasma concentration among the participant with higher BMI. We demonstrated that the correlations between usual intake of these carotenoids assessed by FFQ and their respective plasma concentrations are changed when the participants are grouped by BMI category. Although correlation coefficients were mostly unchanged when they were controlled for BMI in partial correlation analysis, the correlations were higher in the elderly subjects with BMI < 25 than in those with BMI ≥ 30, especially for α-carotene, β-carotene and lutein + zeaxanthin. The present study also shows that, on average, correlations were substantially better in men than women, particularly for vitamin C.

Overall, the correlations shown in the present study for the complete study group as well as for men and women were within the range of those observed in other studies (Jacques *et al.* 1993; Yong *et al.* 1994; Brady *et al.* 1996; Scott *et al.* 1996; Michaud *et al.* 1998; Sasaki *et al.* 2000; El Sohemy *et al.* 2002; Al Delaimy *et al.* 2004) including studies with elderly subjects (Tucker *et al.* 1999; Tangney *et al.* 2004). They tended to be near the lower end for lutein + zeaxanthin, lycopene, and, to a lesser extent, for α-carotene, β-carotene and β-cryptoxanthin, and at the upper end for vitamin C. Compared to several studies that provided diet and serum carotenoid correlations over a full range of BMI values, the correlations among our subjects with BMI < 25 were similar (VandenLangenberg *et al.* 1996; Tangney *et al.* 2004), or slightly higher (Neuhouser *et al.* 2001; Irwig *et al.* 2002; Bermudez *et al.* 2005), except for β-cryptoxanthin and lutein + zeaxanthin which were lower in the present study (VandenLangenberg *et al.* 1996; Neuhouser *et al.* 2001; Irwig *et al.* 2002; Bermudez *et al.* 2005). In contrast, the correlations in the present study were lower in subjects with BMI 25–30 and BMI ≥ 30 (VandenLangenberg *et al.* 1996; El Sohemy *et al.* 2002; Irwig *et al.* 2002; Bermudez *et al.* 2005).

In the multivariate regression analysis of the present study, we demonstrated that after controlling for other variables, the BMI was inversely associated with the plasma concentrations. Furthermore, we showed that all carotenoid plasma concentrations of elderly subjects decreased with increasing BMI, with the lowest concentrations in subjects with BMI ≥ 30.

The finding of lower carotenoid plasma concentrations with increasing BMI can be supported by the findings of Neuhouser *et al.* (2001) who have shown that participants with BMI ≥ 30 had serum nutrient concentrations 2–10 % lower than participants with BMI < 25 and that obesity was a significant predictor of α-carotene plasma concentration. Other studies have also found BMI to be negatively associated with plasma and adipose tissue carotenoid concentrations (Ascherio *et al.* 1992; Brady *et al.* 1996; Scott *et al.* 1996; Vogel *et al.* 1997; Wallstrom *et al.* 2001; Galan *et al.* 2005). In addition to the results of these cross-sectional studies, Andersen *et al.* (2006) have demonstrated, in the only prospective study published to date, that BMI predicted the evolution of serum carotenoids during a 7-year follow-up in the CARDIA study among young non-smoking adults with the exception of lycopene. The authors attributed the lack of relation between BMI and the 7-year change in carotenoids in smokers to the substantially lower plasma levels of smokers compared to non-smokers, except lycopene, for which they did not observe an inverse association with BMI. Other cross-sectional studies have shown that plasma concentrations of β-cryptoxanthin and lutein + zeaxanthin were not affected by BMI (Tucker *et al.* 1999; Al Delaimy *et al.* 2005).

Lower carotenoid concentrations with increasing BMI can be explained by several mechanisms. One hypothesis is that carotenoids are distributed between plasma and adipose tissue, adipose tissue being the dominant storage tissue in man (van Vliet, 1996). Accordingly, a person with high fat mass would have a larger proportion of ingested carotenoids absorbed by fat tissue than would a lean person if all other metabolic factors were equal. This hypothesis can be supported by Yeum *et al.* (1998) who demonstrated that older women with high fat mass showed significant and inverse correlations between anthropometrical measurements such as BMI and fat mass and baseline carotenoid concentrations. Another explanation for the lower carotenoid plasma

Table 6. Predictors of plasma carotenoids and vitamin C (μmol/l) in multivariate analysis in Spanish elderly subjects†

	α-Carotene		β-Carotene		Lutein + zeaxanthin		Lycopene		β-Cryptoxanthin		Vitamin C	
Independent variable	β	SE	β	SE	β	SE	β	SE	β	SE	β	SE
Intercept	4·0*	1·9	3·80*	1·9	4·04*	1·59	11·79****	2·84	5·1*	2·3	5·53****	1·26
Age (years)	−0·01	0·01	−0·01	0·01	0·002	0·006	−0·01	0·01	−0·003	0·01	−0·01	0·01
Sex‡	0·3*	0·12	0·27*	0·12	−0·04	0·10	0·34	0·18	0·02	0·14	0·21*	0·08
BMI (kg/m^2)	−1·34****	0·28	−1·09****	0·28	−0·72**	0·24	−1·87**	0·41	−0·58	0·31	0·03	0·19
Alcohol intake‡	−0·13*	0·06	−0·14*	0·06	0·05	0·05	0·03	0·09	0·001	0·07	−0·06	0·04
Supplement intake	−0·15	0·17	−0·11	0·17	0·01	0·14	−0·27	0·25	0·03	0·19	−0·19	0·11
Smoking habit‡	0·02	0·12	−0·05	0·12	−0·08	0·10	−0·02	0·18	−0·12	0·13	−0·18*	0·08
Fasting state‡	−0·15	0·12	−0·07	0·12	−0·15	0·10	−0·14	0·17	−0·11	0·13	−0·03	0·08
Plasma cholesterol (mmol/l)	0·60**	0·22	0·38	0·22	0·38*	0·19	0·23	0·33	0·34	0·24	–	–
Energy intake (kJ/d)	−0·31	0·17	−0·32	0·17	−0·58****	0·14	−0·89**	0·26	−0·73****	0·21	−0·43**	0·13
α-Carotene intake (mg/d)	0·23****	0·05										
β-Carotene intake (mg/d)			0·32****	0·08								
Lutein + zeaxanthin intake (mg/d)					0·16**	0·06						
Lycopene intake (mg/d)							0·41****	0·11				
β-Cryptoxanthin intake (mg/d)									0·35****	0·07		
Vitamin C intake (mg/d)											0·48****	0·07
Model 1 R^2§	0·147		0·123		0·064		0·088		0·069		0·208	
Model 2 R^2§	0·161		0·128		0·072		0·089		0·069		0·211	
P (test of interaction)§	0·003		0·071		0·034		0·458		0·986		0·178	

*$P<0·05$; **$P<0·01$; ****$P<0·0001$.

† All analyses were performed with log *e*-transformed data for age, BMI, plasma cholesterol, energy intake, intakes of carotenoids and vitamin C, plasma concentrations of carotenoids and vitamin C.

‡ Categorical variables: sex (two categories: men, women), alcohol intake (three categories: 0, 0·1–11·9, >12 g/d alcohol), supplement intake (two categories: yes, no), smoking habit (two categories: ex/non-smoker, current smoker), fasting state (two categories: fasting state, non-fasting state).

§ Model 1, R^2 from main effect models. Model 2, R^2 from main effect models plus an interaction term between BMI and every nutrient intake. *P* value of interaction tests.

concentrations is a higher oxidative stress in persons with increased BMI which has been demonstrated by several studies (Keaney *et al.* 2003; Morrow, 2003; Andersen *et al.* 2006; Weinbrenner *et al.* 2006). Plasma carotenoid concentrations could be decreased to defend the organism from the increased oxidative stress. In the present study, we did not analyse oxidative stress factors to investigate this hypothesis. A third explanation could be that a lower BMI is related to a healthier lifestyle which also increases carotenoid concentrations in plasma, such as a higher physical activity and a higher consumption of fruits and vegetables.

Thus, we could explain the lower carotenoid plasma concentrations observed in the present study with a lower intake of specific foods with a high content of carotenoids. However, the mean intake of the carotenoids as well as of fruit and vegetables did not differ by BMI category, and the mean intake of lutein + zeaxanthin was even significantly higher among the upper BMI categories. In the multivariate regression analysis of the present study, we demonstrated that controlling for other variables, all carotenoid intakes were direct predictors of their respective plasma concentrations. An important additional finding of the present study was that when we added interaction terms between BMI and every nutrient intake in the regression analyses, significant interactions for α-carotene and lutein + zeaxanthin and, to a lower extent for β-carotene ($P=0{\cdot}071$), were observed. The present results lead us to the hypothesis that the plasma concentrations of these three carotenoids could be affected by the interaction of BMI and dietary intake. This finding can explain why the correlations between plasma concentration of α-carotene and β-carotene and their dietary intakes were reduced in the elderly participants of the present study with BMI 25–30 and BMI ≥ 30. This interaction might indicate that a person with BMI ≥ 30 has to consume a higher amount of α-carotene or β-carotene to reach the same plasma concentration as a subject with BMI < 25. In the case of the association between dietary and plasma lutein + zeaxanthin, the correlation was negligible in participants with BMI ≥ 30 which may suggest a null influence of lutein + zeaxanthin intake on its plasma concentrations in obese people.

The present results showing that the associations between dietary and plasma concentrations of α-carotene, β-carotene and lutein + zeaxanthin are influenced by BMI can be supported by Tucker *et al.* (1999) who have shown that the correlations between carotenoid intakes and plasma concentrations improved after adjustment for BMI, plasma cholesterol concentrations and smoking status, particularly for α- and β-carotene, in men, but not in women.

The question of whether the mechanisms which are responsible for the lower concentrations of α-carotene, β-carotene and lutein + zeaxanthin on the one hand, and lycopene and β-cryptoxanthin on the other, in people with BMI ≥ 30 are different, cannot be answered by the present study. Further studies which investigate the metabolism of carotenoids and the causes of lower plasma concentrations in obese people are needed.

Concerning vitamin C, we could demonstrate that the correlation coefficients were higher in men than in women, but that the adjustment for age, BMI, smoking, alcohol and supplement intake and fasting state decreased the coefficient considerably in men but not in women. This can be explained by the higher percentage of current smokers in the group of men compared to women and the higher alcohol intake by men. Both factors are known to influence vitamin C concentrations in plasma (Galan *et al.* 2005). Furthermore, we showed that the correlation between vitamin C intake and plasma concentration was lowest in elderly subjects with BMI 25–30 and BMI ≥ 30 compared to subjects with BMI < 25. In accordance with the hypothesis for the lower correlations of carotenoids in subjects with BMI ≥ 25, we might suppose that also vitamin C concentrations were lower in these subjects and did not reflect their dietary intake. Galan *et al.* (2005) have found lower vitamin C concentration in obese participants. But in the present study, neither the vitamin C intake nor the plasma concentrations substantially differed between BMI categories. In addition, BMI was not a significant predictor for plasma vitamin C concentration in the present study, in contrast to the study of Galan *et al.* (2005). Furthermore, the interaction term of dietary vitamin C intake and BMI was not significant in the multivariate regression analysis. Thus, new studies would be needed to explore further the relationship between BMI and vitamin C, especially in elderly individuals.

One limitation of the study was the non-fasting state of 85 % of the elderly participants when blood samples were taken. However, since all participants were advised to avoid carotenoid-rich foodstuffs in the meal prior to sampling, it is unlikely that TAG-rich chylomicrons would contain significant amounts of carotenoids, and serum carotenoids reside mainly in the cholesterol-rich lipoproteins. In view of this, we consider the correction of carotenoids for cholesterol as the correct approach in the present study. All analyses were adjusted for fasting state and the comparison of the data of carotenoid and vitamin C intake and the plasma concentrations of non-fasting and fasting participants did not show significant differences among the groups.

From the results of the present study, we suggest that both the reported dietary intake and the analysed plasma concentrations of carotenoids were mainly correct, and that given a constant daily carotenoid intake, this would not result in similar plasma concentrations in elderly subjects according to their BMI regarding α-carotene, β-carotene and lutein + zeaxanthin. If this is true, more research is needed as this may help more accurate interpretation of study results exploring the relationships between carotenoids and disease outcomes, particularly for those diseases (e.g. age-related macular diseases) for which the lack of consistency is still evident. Therefore, in order to explore the association between the carotenoid intake and the risk of disease, it seems important not only to examine the effect of dietary intake as assessed by dietary questionnaires but also to analyse plasma concentrations. In those studies with only plasma concentration data, it may well be justifiable to stratify analysis by BMI categories in order to detect a possible effect modification.

In conclusion, the present results showed that, in general, the dietary intakes of several carotenoids and vitamin C assessed by a FFQ were significant determinants of their respective plasma concentrations in an elderly Spanish population. However, the magnitude of the diet–plasma correlations was in the lower range of correlations found by other

studies except for vitamin C which was similar. We also showed that BMI was negatively associated with plasma concentration of carotenoids and found significant interactions between BMI and the intakes of α-carotene, β-carotene and lutein + zeaxanthin, producing higher plasma–diet correlations of these three carotenoids in subjects with BMI < 25, and much lower in subjects with BMI ≥ 30, where correlation was indeed negligible for lutein + zeaxanthin.

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