

## Age-related changes in plasma lycopene concentrations, but not in vitamin E, are associated with fat mass

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(Received 28 June 1999 – Revised 22 February 2000 – Accepted 21 March 2000)

The aim of the present study was to assess the influence of age on plasma concentration of  $\alpha$ -tocopherol, retinol and carotenoids with a special attention paid to natural differences in body composition. Forty healthy subjects were recruited: twenty were less than 35 years old and twenty above 60 years old. Males and females were equally represented in each age group. Subjects were kept in energy balance and received controlled diets for 36 h. Fat mass and fat-free mass were determined with the <sup>18</sup>O-enriched water dilution technique. Plasma vitamins A and E, and carotenoid levels were determined after 12 h fasting and were shown to be similar in women and men. Plasma  $\alpha$ -tocopherol concentration increased with age (+44 % elderly *v.* young), and correlated with % fat mass and plasma cholesterol. After adjustment for plasma cholesterol, the effect of age and % fat mass disappeared. In contrast, plasma lycopene level was 2-fold lower in the elderly than in the young group, and was inversely correlated with fat mass. When lycopene values were adjusted for fat mass, the effect of age disappeared. These results suggest that plasma levels of vitamin E and lycopene differed in the two age groups and that differences in plasma cholesterol and fat mass might participate in such an effect. Short-term vitamin intake did not appear to influence plasma vitamin concentrations.

### Age: Carotenoids: Vitamin E: Body composition

Carotenoids and vitamin E are involved in defences against free radical reactions which might play a key role in the ageing process and age-related diseases (Harman, 1994). Carotenoid and vitamin E status, as assessed from plasma concentrations, do not seem to be impaired in healthy elderly subjects (Battisti *et al.* 1994; Hallfrisch *et al.* 1994).

However, age is associated with an increase in fat mass (FM) and a decrease in fat-free mass (FFM) mainly in the form of muscle (Cohn *et al.* 1980). In man, more than 90 %  $\alpha$ -tocopherol ( $\alpha$ -T) is stored in adipose tissue (Traber & Kayden, 1987). Due to the similar concentrations of carotenoids in liver (2.7  $\mu$ g/g wet tissue) and in adipose tissue (1.8  $\mu$ g/g wet tissue), and because body FM represents 20–25 % body weight, FM is likely to be a

large carotenoid store (Parker, 1988; Kaplan *et al.* 1990). Furthermore, FM is about 30 % in males and 36 % in females 80 years of age (Cohn *et al.* 1980). This might increase the carotenoid store.

The question arises as to whether body FM modulates the blood vitamin E and carotenoids concentrations in elderly subjects.

Supplementation studies in healthy subjects have shown that adipose tissue accumulates  $\beta$ -carotene (BC) (Johnson *et al.* 1995) and vitamin E (Brouwer *et al.* 1998). However, it is not clear whether adipose tissue mass affects plasma vitamin E concentration. When fed a vitamin E-deficient diet, rats showed a decrease in plasma and liver  $\alpha$ -T (Machlin *et al.* 1979), while in sheep concentrations

**Abbreviations:** BC,  $\beta$ -carotene; FFM, fat-free mass; FM, fat mass;  $\alpha$ -T,  $\alpha$ -tocopherol.

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decreased at the same rate in muscle and adipose tissue (Fry *et al.* 1993). In human subjects, a 17 h fast, which resulted in significant lipolysis, did not increase plasma  $\alpha$ -T and BC (Brouwer *et al.* 1998).

When accurate methods were used for body composition estimates, conflicting data were observed. In elderly men, Zhu *et al.* (1997) reported that the increase in plasma BC concentration in response to BC supplementation was inversely related to FFM, but not to FM. Yeum *et al.* (1998) showed an inverse association between FM, or FFM, and plasma BC only in women but not in men. Furthermore, plasma BC response to a carotenoid-rich diet was inversely related to FFM in old women (Yeum *et al.* 1998), while no correlation was found in young subjects.

The aim of the present study, therefore, was to examine the effect of age on plasma concentration in  $\alpha$ -T, retinol and carotenoids with special attention paid to natural differences in body composition as FM and FFM.

## Materials and methods

### Subjects

Forty healthy non-smoking subjects were recruited after a full medical examination. They had been weight-stable (within 1 kg) for the last 2 months. None was taking vitamin supplements. None of them was suffering from diabetes, none was taking drugs known to influence energy or macronutrient metabolism. They all had a normal plasma lipid profile (total cholesterol, triacylglycerol). Subjects with a BMI > 25 (young subjects) or 30 kg/m<sup>2</sup> (elderly subjects) were excluded from the study. Subjects gave informed written consent for participating in the study. The study protocol was approved by the local ethics committee and by the French Ministry of Health.

Subjects were separated into young ( $n$  20, age < 35 years) and elderly ( $n$  20, age > 60 years); 60 years corresponds to retirement in France, and 35 years was chosen so that the two groups were separated by a wide age-gap. There was an equal number of men and women in each age group. Physical characteristics for subjects are given in Table 1.

### Study protocol

Subjects stayed in the metabolic suite of the Human Nutrition Laboratory (Université d'Auvergne, Clermont-Ferrand, France) for 36 h for an investigation of their energy expenditure. They were kept in close energy balance ( $-4.4$  (SE 8.7) % energy expenditure).

They stayed in bed from 23.00–07.00 hours, and ate at 08.00, 12.30 and 19.00 hours. They walked on a treadmill for 30 min at 10.00, 11.30, 15.00 and 16.30 hours. They practised weightlifting for 20 min at 18.00 hours. In-between these events, subjects were free to do light activities (reading books, listening to the radio, watching television) but were not allowed to rest in bed or to have extra exercise. The diet provided 50 % energy from carbohydrates, 35 % from lipids and 15 % from protein.

Body composition was measured while the subjects stayed in the room calorimeter with the <sup>18</sup>O-dilution technique described by Vaché *et al.* (1995). Briefly, subjects gave a baseline urine sample at 23.00 hours, drank a weighed amount of <sup>18</sup>O-enriched water (Cortec, Paris, France) (2 % enrichment, about 50 g;...) and went to bed. They were woken at 06.00 and 07.00 hours the following morning to provide a urine sample. Total body water calculations were performed from the net increase in <sup>18</sup>O enrichments above background values (Vaché *et al.* 1995). FFM was calculated as total body water divided by the hydration factor, a value not affected by age (Ritz, 1998). FM was computed as the difference between body weight and FFM. The percentage of body fat is FM/body weight.

### Biochemical analyses

After 12 h fasting (36 h after entering the facility), 10 ml blood was drawn to perform measurements of plasma triacylglycerol, cholesterol, free-fatty acid, vitamin, and carotenoid concentrations.

Free-fatty acid concentrations were measured using a specific enzymatic assay (Unipath, NEFAC, Dardilly, France) and absorbances were read at 550 nm on a Uvikon 810 spectrophotometer (Kontron, Lyon, France). Plasma retinol,  $\alpha$ -T and carotenoids were extracted twice by hexane and assayed using a reverse-phase HPLC apparatus

**Table 1.** Physical characteristics of the subjects  
(Mean values with their standard errors)

	Young				Elderly				Statistical significance of difference between means (two-way ANOVA)	
	Men ( $n$ 10)		Women ( $n$ 10)		Men ( $n$ 10)		Women ( $n$ 10)		Age	Sex
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Age (years)	24.4	1.0	25.0	1.5	64.3	1.4	64.8	1.0	$P < 0.001$	NS
Weight (kg)	69.7	2.1	59.7	2.5	74.6	3.0	62.0	1.8	NS	$P < 0.001$
Height (cm)	173.6	1.2	162.3	1.9	171.7	1.4	155.2	2.0	NS	$P < 0.001$
BMI (kg/m <sup>2</sup> )	23.3	0.9	22.6	0.8	25.3	0.9	25.8	0.8	$P < 0.01$	NS
Fat mass (%)	15.2	2.1	23.6	2.0	22.5	1.5	33.9	1.6	$P < 0.001$	$P < 0.001$
FFM (kg)	64.0	1.6	45.8	1.1	54.7	1.7	38.6	0.8	$P < 0.001$	$P < 0.001$
Plasma cholesterol (mmol/l)	5.21	0.20	5.07	0.31	5.78	0.22	6.80	0.20	$P < 0.001$	NS
Plasma triacylglycerol (mmol/l)	1.01	0.10	1.07	0.12	1.17	0.11	1.20	0.12	NS	NS
Plasma free-fatty acids ( $\mu$ mol/l)	499	47	523	52	451	38	494	82	NS	NS

**Table 2.** Plasma concentrations ( $\mu\text{mol/l}$ ) of retinol,  $\alpha$ -tocopherol and carotenoids in young and old women and men\* (Mean values with their standard errors)

	Young				Elderly				Statistical significance of difference between means†		
	Men ( <i>n</i> 10)		Women ( <i>n</i> 10)		Men ( <i>n</i> 10)		Women ( <i>n</i> 10)		Age (A)	Sex (S)	A $\times$ S
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Retinol	1.96	0.13	2.01	0.22	1.76	0.14	1.98	0.18	NS	NS	NS
$\alpha$ -Tocopherol	18.24 <sup>b</sup>	1.69	19.08 <sup>b</sup>	1.42	26.33 <sup>a</sup>	2.87	21.77 <sup>ab</sup>	1.81	$P = 0.019$	NS	NS
Lutein	0.39	0.07	0.34	0.07	0.48	0.09	0.40	0.05	NS	NS	NS
$\beta$ -Cryptoxanthin	0.18	0.04	0.23	0.10	0.14	0.04	0.24	0.09	NS	NS	NS
Lycopene	0.18 <sup>a</sup>	0.04	0.17 <sup>a</sup>	0.04	0.09 <sup>b</sup>	0.01	0.09 <sup>b</sup>	0.02	$P = 0.004$	NS	NS
$\alpha$ -Carotene	0.07	0.02	0.05	0.02	0.08	0.02	0.07	0.03	NS	NS	NS
<i>trans</i> - $\beta$ -Carotene	0.37	0.13	0.19	0.06	0.26	0.07	0.27	0.09	NS	NS	NS
13- <i>cis</i> - $\beta$ -Carotene	0.02	0.01	0.01	0.01	0.01	–	0.01	–	NS	NS	NS

<sup>a,b</sup>Group mean values within a row with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Young, <35 years old; old, >60 years old; for details of subjects see Table 1.

† Two-way ANOVA and Fisher's protected least significant difference test. Values were log-transformed to improve normality before statistical evaluation.

(Kontron series 400, Kontron) with retinyl laurate, tocopherol acetate and echinenone as internal standards (Grolier *et al.* 1998). Retinol and  $\alpha$ -T were eluted on a Nucleosil column (250  $\times$  4.6 mm; Interchim, Montluçon, France) with methanol as mobile phase (2 ml/min). Carotenoids were analysed using a Vydac TP 54 column (250  $\times$  4.6 mm) and a Nucleosil column (150  $\times$  4.6 mm; Interchim) in series, and a mixture of acetonitrile–methanol (65:35, v/v) containing 50 mM-ammonium acetate–dichloromethane–double-distilled water (70:15:10:5, by vol.), as mobile phase (2 ml/min). Retinol,  $\alpha$ -T and carotenoids were detected at 325, 292 and 450 nm respectively, and were quantified using correction by yield internal standard and calibration with external standard.

### Statistics

Results are expressed as mean values with their standard errors. Data were log transformed to make the distributions normal. Comparison between groups was made by two-way ANOVA, with age and sex as categories. Fisher's protected least significant difference tests were performed for the comparison of means. Correlations were sought with a Pearson correlation test. Adjustments were performed as

described in Ravussin & Bogardus (1989). Significance was accepted at the 5 % level. All computations were performed with Statview 4.0 statistical package (Abacus Concepts Inc., Berkeley, CA, USA).

### Results

No significant effect of sex was observed for any of plasma vitamins or carotenoid concentrations (Table 2). A significant effect of age was shown on  $\alpha$ -T ( $P = 0.019$ ), elderly women having the highest concentrations (Table 2). Plasma  $\alpha$ -T was significantly affected by age ( $P = 0.019$ ) and correlated with % FM ( $r +0.38$ ) and plasma cholesterol ( $r +0.70$ ). However, no correlation was found between plasma  $\alpha$ -T and BMI, FFM or FM. Plasma cholesterol was significantly affected by age ( $P < 0.001$ ). After adjustment for plasma cholesterol, there remained no effect of age or % FM on plasma  $\alpha$ -T concentration.

Lycopene concentrations were 50 % lower in the elderly group than the young group ( $P = 0.004$ ). Plasma lycopene was inversely correlated with BMI ( $r -0.38$ ), % FM ( $r -0.40$ ), and FM ( $r -0.48$ ). After adjustment for differences in FM, plasma lycopene concentrations were no longer different between the two age groups.

Plasma  $\alpha$ -carotene, BC and  $\beta$ -cryptoxanthin were not

**Table 3.** Intake of lipids and vitamins during a 36 h stay in the Human Nutrition Laboratory by young and old men and women\*

	Young				Elderly				Statistical significance of difference between means†		
	Men ( <i>n</i> 10)		Women ( <i>n</i> 10)		Men ( <i>n</i> 10)		Women ( <i>n</i> 10)		Age (A)	Sex (S)	A $\times$ S
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Lipid (g/d)	75.4	2.3	97.3	2.2	75.7	1.2	100.0	2.7	NS	$P < 0.0001$	NS
Lipid (% EI)	34.6	0.2	34.1	0.2	34.4	0.2	34.6	0.4	NS	NS	NS
Cholesterol (mg/d)	364	12	498	18	376	6	490	12	NS	$P < 0.0001$	NS
Vitamin E (mg/d)	5.5	0.24	7.1	0.34	5.7	0.17	7.3	0.18	NS	$P < 0.0001$	NS
Total carotene ( $\mu\text{g/d}$ )	947	49	1242	122	862	64	1232	30	NS	$P < 0.001$	NS
$\beta$ -Carotene ( $\mu\text{g/d}$ )	732	58	1149	189	645	60	897	50	NS	$P = 0.002$	NS
Dietary fibre (g/d)	15.4	0.5	18.8	0.8	15.1	0.4	21.2	0.5	NS	$P < 0.0001$	$P = 0.03$

EI, energy intake.

\* Young, <35 years old; old, >60 years old. The diet provided 50 % energy from carbohydrate, 35 % from lipids and 15 % from protein. The subjects were kept in energy balance ( $-4.4$  (SE 8.7) % energy expenditure). For details of subjects see Table 1.

† Two-way ANOVA and Fisher's protected least significant difference test. Values were log-transformed to improve normality before statistical evaluation.

correlated with any of anthropometric or plasma variables measured. Significant correlations were obtained between retinol and plasma free-fatty acids ( $r$  0.32) and between lutein and cholesterol ( $r$  0.40).

Table 3 shows the intakes of fat, vitamin E and carotene for each group during the 36 h stay in the laboratory. They were significantly affected by sex but not by age.

### Discussion

The present study has shown that absolute plasma concentrations of  $\alpha$ -T were higher in elderly than in young persons, while the reverse was true for plasma lycopene. When adjusted for confounding variables (cholesterol for  $\alpha$ -T, and FM for lycopene, both of which increased with age) there remained no effect of age. In the subjects studied here, age did not appear to influence plasma carotene concentrations in an independent way.

Data from the literature show that plasma  $\alpha$ -T appears to be increased with age in most longitudinal (Haller *et al.* 1996; Öhrvall *et al.* 1996) and cross-sectional studies (Battisti *et al.* 1994; Hallfrisch *et al.* 1994; Borel *et al.* 1997; Winklhofer-Roob *et al.* 1997). In some cases, the increase is limited to women (Ascherio *et al.* 1992) or to middle-age subjects (Knekt *et al.* 1988). Few studies found no effect of age (Morinobu *et al.* 1994; Alberti-Fidenza *et al.* 1995) or even a decrease in plasma  $\alpha$ -T concentrations with age (Vatassery *et al.* 1983; Mino *et al.* 1993).

FM, which is a significant store for  $\alpha$ -T (Parker, 1988; Kaplan *et al.* 1990), can be estimated with the BMI. Accurate estimates of FM can be achieved with the  $^{18}\text{O}$ -dilution technique (Ritz, 1998). The positive association between plasma  $\alpha$ -T and BMI is found in some (Patterson *et al.* 1993; Su *et al.* 1998) but not all studies (Ascherio *et al.* 1992; Coudray *et al.* 1997). On the other hand, plasma  $\alpha$ -T is correlated with plasma cholesterol (Thurnam *et al.* 1986, Borel *et al.* 1997). The data reported here show that with an adequate estimate of body composition, there is no relationship between FM or FFM and  $\alpha$ -T. The correlation is with adiposity (% FM) and not FM. Plasma  $\alpha$ -T was related to plasma cholesterol, and after taking this variable into account no effect of age could be seen. Therefore, as suggested by others (Battisti *et al.* 1994; Winklhofer-Roob *et al.* 1997) age and/or FM do not have a genuine effect on plasma  $\alpha$ -T. Furthermore, although mathematically a large store of  $\alpha$ -T (concentration  $\times$  mass), adipose tissue mass may not contribute directly to plasma  $\alpha$ -T. This had been suggested by studies with vitamin E-deficient diets (Machlin *et al.* 1979; Fry *et al.* 1993) and studies on fasting human subjects (Brouwer *et al.* 1998). Plasma concentrations of  $\alpha$ -T are therefore likely to be under complex influences. The present study did not report vitamin intakes over a long period. However, data acquired during the stay in the metabolic suite while subjects were in energy balance show that the intake of vitamin E was similar in elderly and in young subjects. This suggests that dietary intake might not explain increased plasma  $\alpha$ -T in the elderly, but that cholesterol metabolism, which is affected by age (Miller, 1984; Ribaya-Mercado *et al.* 1995), is more likely to be a significant determinant. This is no proof that long-term intake of vitamin E has an effect on

plasma  $\alpha$ -T or not. However, energy needs decrease with age (Carpenter *et al.* 1995), and fat intake appears to be either unchanged or decreased in elderly persons (Morley, 1997; Euronut-SENECA investigators, 1996). Moreover, the correlation between vitamin A and E intake and plasma concentrations is weak (Euronut-SENECA investigators, 1991; Willett *et al.* 1983; Stryker *et al.* 1988). Therefore, we can assume that long-term intake of vitamin E might not play a great role in the increase in plasma  $\alpha$ -T.

Inconsistent relationships were found between plasma carotenoids and age or BMI (Ringer *et al.* 1991; Fernandez-Banares *et al.* 1993; Järvinen *et al.* 1993; Hallfrisch *et al.* 1994; Johnson *et al.* 1995; Vogel *et al.* 1997; Winklhofer-Roob *et al.* 1997; Zhu *et al.* 1997). Even when direct measurements of body compartments were performed, some studies suggest a negative relationship between FFM or FM and plasma carotenoids (Zhu *et al.* 1997) while other studies showed no relationship (Henderson *et al.* 1989; Ringer *et al.* 1991). The effects of supplementation with carotenoids appears to be influenced by FFM but not by FM (Yeum *et al.* 1998). The present study found no correlation between either FFM or FM and any of the plasma carotenoid levels. As for vitamin E, carotenoid intakes during controlled conditions were affected by sex, but not by age. Borel *et al.* (1998), studying subjects from the same geographical area, found no difference between young and elderly persons regarding vitamin A and  $\beta$  carotene intakes. The comments made earlier about long-term intake of vitamins also apply to carotenoids.

Lycopene was unique in that its concentration was negatively related to body compartments, and plasma concentrations were lower in elderly than in young subjects. Most studies in the literature also found such a negative association (Kaplan *et al.* 1990; Ascherio *et al.* 1992; Peng *et al.* 1995; Brady *et al.* 1996; Michaud *et al.* 1998). Very few studies found a positive (Winklhofer-Roob *et al.* 1997) or no relationship (Ringer *et al.* 1991; Vogel *et al.* 1997; Yeum *et al.* 1998). The present study shows that when plasma lycopene is adjusted for FM, no effect of age can be demonstrated. It suggests that FM plays an important role in lycopene metabolism, although it cannot be simply taken as a reservoir, since there is a negative association between both variables. Our study could not highlight the relationship between intake in lycopene and plasma concentrations. In our study the plasma lycopene levels were much lower than those determined in the two other American studies (Zhu *et al.* 1997; Yeum *et al.* 1998). Similar low levels were previously reported in young French individuals (Borel *et al.* 1997) and in old subjects from Portugal, Spain and Poland (Haller *et al.* 1996). Such plasma differences might reflect regional aspect of tomato consumption and might impede correlation between plasma lycopene and body composition.

In conclusion, this study suggests that age (*per se*) might only be a weak determinant of plasma vitamin A and E concentrations. Other variables affected by age (such as cholesterol concentration and FM) appear to be more significant determinants. However, adipose tissue should not be considered as an inert vitamin store, just because it is large. Further studies are required to delineate how plasma

concentrations of vitamin A and E are affected by fat metabolism (cholesterol and triacylglycerol in adipose tissue) and by intake.

### Acknowledgements

We thank Line Godiveau for secretarial assistance and Marthe O'Aconnar for technical help.

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