Comparison of the postprandial chylomicron carotenoid responses in young and older subjects

Nicolas Cardinault, Viviane Tyssandier, Pascal Grolier, B.M. Winklhofer-Roob, J. Ribalta, Corinne Bouteloup-Demange, Edmond Rock, Patrick Borel

Comparison of the postprandial chylomicron carotenoid responses in young and older subjects.

Nicolas Cardinault [1], Viviane Tyssandier [1], Pascal Grolier [1], Brigitte M. Winklhofer-Roob [2], Josep Ribalta [3], Corinne Bouteloup-Demange [4], Edmond Rock [1], Patrick Borel [1]

[1]Unité Maladies Métaboliques et Micronutriments, INRA, Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle. [2]Institute of Molecular Biology, Biochemistry and Microbiology, Karl-Franzens University, Graz, Austria. [3]Rovira University, Reus, Spain. [4]Unité d'Exploration en Nutrition, Laboratoire de Nutrition Humaine, 58, rue Montalembert, 63000 Clermont-Ferrand. France.

Correspondence :
Patrick.Borel@univ-amu.fr
13385 Marseille Cedex 5, FRANCE

This study has been carried out with financial support from the Commission of the European Communities, specific RTD program "Quality of Life and Management of Living Resources", QLK1-CT-1999-00830. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

Running head: Carotenoid bioavailability in healthy old

Background: The plant carotenoids may contribute to the beneficial health effect of fruits and vegetable rich-diet. Epidemiological studies consistently associated high plasma carotenoids status with reduce age-related diseases. However, the data concerning the bioavailability of carotenoids in older are scarce. **Objective**: To test whether there is an age effect on carotenoid bioavailability **Design**: Eight young (20-35 y) and eight older (60-75 y) healthy adults ingested three different meals containing 40 g triacylglycerols (TG) and vegetable sources of carotenoids. These sources were either 188 g carrot purée which provided 30 mg beta-carotene as the main carotenoid, or 61 g tomato purée providing 30 mg lycopene, or 260 g cooked chopped spinach providing 30 mg lutein. TG and carotenoids were assayed in chylomicrons (CM) collected for 9 h postprandially. **Results**: There was no major effect of age on the postprandial CM TG response (0-9 h area under the curve (AUC)). There was no major effect of age on the post-prandial CM all-*trans* beta-carotene, *cis* beta-carotene, alpha-carotene, and lutein responses. Adjustement of these responses by the CM TG responses did not reveal any age effect. While there was no significant effect of age on the CM lycopene response, the CM TG-adjusted lycopene response was significantly lower (-40%) in the old than in the young ($P < 0.04$). The *cis*-*trans* ratios of CM beta-carotene was not significantly different between the old and the young subjects. There was no significant effect of age on the ratio of CM retinyl-palmitate to the sum of alpha-carotene and beta-carotene measured after the carrot meal. **Conclusions**: The bioavailability of lycopene is apparently impaired in the old, while there is no major difference in the bioavailability of beta-carotene, alpha-carotene and probably lutein. There is also no major effect of age on the *cis*-*trans* isomerization of beta-carotene during absorption, and in the intestinal conversion of provitamin A carotenoids into vitamin A.

Keywords: carotenoids . vitamin A . *cis*- beta-carotene . provitamin A activity . aging . postprandial response

Introduction

Epidemiological studies consistently associate diets rich in fruit and vegetables with a reduced incidence of several degenerative diseases [1, 2]. Carotenoids, plant pigments with demonstrated antioxidant properties [3], are assumed to be involved in this effect [4]. The suspected beneficial effect of carotenoids is supported by the fact that high consumption and high plasma concentrations of carotenoids have been associated with a lower incidence of certain cancers [5, 6], cardiovascular diseases [7, 8] and degenerative eye diseases [9, 10], although intervention trials have shown that supranutritional doses of synthetic β-carotene can be harmful in smokers [11, 12]. β-Carotene, lycopene and lutein are the main carotenoids in human diet and tissues. Although they belong to the same pigment family and possess closely similar molecular characteristics, these carotenoids have different biological properties. Of the three mentioned above, only β-carotene has a provitamin A activity. Lycopene is currently the only carotenoid thought to be involved in the prevention of prostate cancer [13]. Lutein, and zeaxanthin, are the only carotenoids assumed to play an important role in the retina [10]. Concomitant to these different biological properties, it has recently been shown that the bioavailability [14, 15] and metabolism [16] of different carotenoid species are different. Taken together these findings show that it is essential to study the individual metabolism of each carotenoid species, to gain a fuller understanding of their fate in the body.

Although some studies have compared fasting plasma carotenoids in young and older subjects [17-19], and one study has shown that gastric acidity, which has a high prevalence in elderly, influences β-carotene blood response to dietary β-carotene [20, 21], there are very few data on the effects of healthy aging on both carotenoid bioavailability [22], and provitamin A activity of carotenoids. Only two studies have addressed this subject, and both studied the bioavailability of purified β-carotene [23, 24]. Yet data on carotenoid metabolism in aged subjects are essential for a better understanding of carotenoids [25] and vitamin A status in the elderly. Such data may also become especially important if the suggested [26] beneficial

health effects of one or more carotenoids in the elderly are firmly demonstrated. Because aging affects numerous physiologic processes, it may directly or indirectly affect carotenoid bioavailability at different levels. First, age-related impairment of gastrointestinal tract functions [27, 28], on which depends the bioavailability of some micronutrients [29], may modify the efficiency of intestinal absorption of carotenoids. Age-related modifications of enterocyte functions may also affect the conversion of provitamin A carotenoids into vitamin A [30] or the *cis-trans* isomerization of carotenoids during absorption [31]. Lastly, age-related modifications of chylomicron metabolism [32] may affect the transport of carotenoids from the intestine to the liver.

The main objective of this work was to determine whether the bioavailability of the three main carotenoids (β-carotene, lycopene and lutein), supplied in their original vegetable matrix is markedly impaired in this growing group of the population. In other words: does carotenoid absorption efficiency markedly change with aging ? To answer this question we compared the postprandial chylomicron carotenoid responses to carotenoid-rich meals in young and old subjects.

Subjects and methods

Study population

Sixteen male, non-obese non-smoking volunteers, with a body mass index between 19 and 29 kg/m^2, were recruited. Eight were young adults (20-35 y) and eight were old adults (60-75 y). The study was approved by the regional committee on human experimentation of the university hospital in Clermont-Ferrand (France). Informed written consent was obtained from each volunteer. The subjects did not take any medication nor dietary supplements either during the month before the study started or during the study period. The subjects were apparently healthy, according to clinical and laboratory examination including fasting plasma lipid and glucose concentrations and disease history. The subjects' usual diet was monitored by means of a 5-d food diary the week before the experiment. This diary was analyzed for nutrient composition using diet analyzer software (GENI; Micro 6, Nancy, France). The database of the software was completed for carotenoids with a carotenoid food-composition database [33]. The volunteers' characteristics are presented in **TABLE 1**.

Analysis of vegetable carotenoids

Vegetables were purchased at a local supermarket (Stoc, Clermont-Ferrand, France). They were purchased from the same lot. A procedure to accurately extract carotenoids from vegetables was drawn up after preliminary experiments. Several combination of solvents were tried, the best combination being the one that gave the whitest vegetable matrix after extraction. The procedure, which was performed under yellow light, was as follows: 50-70 mg vegetables were added with 7 mL methanol containing 0.57 % $MgCO_3$ (Sigma, Saint Louis, USA) and 0.2 μg/mL internal standard (echinenone, Roche Vitamines, France). After homogenization for 30 s with a vortex blender, 7 mL trichloromethane (containing 0.005 % butylated hydroxy toluene as an antioxidant) were added. The sample was homogenised again

for 30 s with the vortex blender. After 15 min. rest, 7 mL distilled water was added. After centrifuging (2,000 x *g*, 10 min., room temperature), the lower phase containing most of the carotenoids was collected. Carotenoids remaining in the upper phase were extracted as follows: 5 mL tetrahydrofuran was added, the mixture was then vortexed for 30 s, and dichloromethane (5 mL) added. It was then vortexed for 30 s, distilled water (3 mL) was added, and it was vortexed again for 30 s. After centrifuging (2,000 x *g*, 10 min., room temperature), the lower phase was collected and pooled with the previously collected phase. After evaporation to dryness under nitrogen, the dried extract was dissolved in 1 mL tetrahydrofurane/acetonitrile/dichloromethane (10/45/45; v/v/v). Carotenoids were quantified by reverse-phase HPLC as described below.

Postprandial experiments

Each subject consumed, in a random order, three different test meals on three different days. Each postprandial experiments were separated by at least two weeks. After the subjects had fasted overnight for 12 h, an antecubital vein was catheterized with an intravenous cannula equipped with disposable obturators (Becton Dickinson, Meylan, France). A baseline fasting blood sample was collected and the subjects ate a test meal within 20 ± 2 minutes. The composition of the meal was: 60 g wheat semolina (cooked and hydrated with 120 mL water), 40 g peanut oil, 2 pieces of bread (45 g), 1 cooked egg white (35 g), 1 serving of yogurt (125 g) and different amounts of vegetables (as natural carotenoid-rich sources). The vegetables were either: 188 g cooked carrot purée (30 mg β-carotene as the main carotenoid and 20.3 mg α-carotene), or 61 g cooked tomato purée (30 mg lycopene as the main carotenoid and 0.54 mg β-carotene), or 260 g cooked chopped spinach (30 mg lutein as the main carotenoid and 11.6 mg β-carotene). The amount of carotenoids provided by the meals was higher than the amount usually consumed in western countries, but it can be attained under certain dietary conditions and it was designed to accurately follow the fate of these micronutrients in the

chylomicron fraction. Before each postprandial experiment, the volunteers were asked to avoid consuming foods rich in carotenoids for three days, by providing them with a list of such foods. The last evening meal before the postprandial experiment was eaten in the Human Nutrition Research Center (HNRC) laboratory of human nutrition (Clermont-Ferrand, France). This meal was carotenoid-free. Blood samples were collected every hour from 2 h to 9 h during the postprandial period. Subjects did not get any food until the end of the postprandial experiment, i.e. after the last blood sample was collected. They were only allowed to drink moderate amount of tap water during the postprandial period.

Chylomicron preparation

Blood was collected in EDTA-treated evacuated tubes and plasma was prepared immediately by centrifugation (910 x g, 4°C, 10 min). Chylomicrons were isolated at the day of blood collection from 5 mL plasma layered under 5 mL 0.9% NaCl by ultracentrifugation (130 000 x *g*, 28 min, 10 °C) in a Kontron (Zurich, Switzerland) SW TST41.14-41000 swinging bucket rotor. It had been verified that this method gives the same results than the method usually done with 2 mL plasma and 3 mL 0.9% NaCl [34-36]. The so-called chylomicron fraction (S_f > 1000) contained chylomicrons plus large chylomicron remnants and, theoretically, few hepatic VLDL. Indeed, VLDL of hepatic origin consist of two major subfractions: VLDL-1 (S_f 60-400) and VLDL-2 (S_f 20-60) [37] with lower S_f than large chylomicrons. Chylomicron fractions were stored at – 80°C under nitrogen until analyzed.

Analytical determinations

Carotenoids were extracted from the chylomicrons using ethanol (containing echinenone as internal standard), that precipitates the proteins, and hexane to solubilize the carotenoids. The hexane extract was evaporated to dryness under nitrogen and the residue was solubilized in an acetonitrile/dichloromethane mixture (1:1, by vol). Carotenoids were

quantified by reverse-phase HPLC on a Waters system (Waters SA, Saint-Quentin en Yvelines, France). This system comprised a Waters 660 pump, a Waters 717 plus cooled auto-sampler, and a Waters 996 UV-visible diode-array detector. Carotenoids were separated using two columns set in series [38]: a 150 × 4.6 nm RP C_{18}, 3-µm Nucleosyl (Interchim, Montluçon, France), coupled with a 250 × 4.6 nm RP C_{18}, 5-µm Vydac TP54 (Hesperia, CA, USA), and a Hypersil guard column. The mobile phase was an isocratic acetonitrile: dichloromethane: methanol (containing 50 mmol/L ammonium acetate): water (70:10:15:5, by vol) mixture. Carotenoids were detected at 450 nm and identified by comparison of their retention time and spectral analysis (from 300 to 550 nm) with those of pure (> 95%) standards of the following carotenoids: lutein, echinenone, *all-trans* lycopene, α-carotene, *all-trans* β-carotene and *13-cis* β-carotene (Roche Vitamines France, Neuilly-sur-Seine, France). In this system the *cis*-isomers of lycopene were not accurately separated from the *all-trans* isomer, thus all lycopene isomers were quantified together. Quantification was conducted using MILLENIUM 32 Waters software (version 3.05.01). Internal standard allowed to calculate and overall recovery yield 75-100%. All the solvents used for carotenoid analyses were HPLC graded from Carlo Erba (Chaussée de Vexin, France).

Triacylglycerols were assayed in chylomicrons by using an enzymatic colorimetric method with a commercial kit (Biomerieux, Craponne, France). The concentrations were measured spectrophotometrically at 490 nm by using a Microplate Reader MR 700 (Dynatech Laboratories Inc, Guernsey, UK).

Statistical analysis

Power analysis: because there was no available data on the effect of age on the postprandial chylomicron carotenoid response we used previous results on the effect of age on vitamin E chylomicron response [39] to perform the power analysis. These results showed that we could expect a 50% decrease in the chylomicron micronutrient response (AUC) between

young and old. Assuming a SD of 40% of the mean value in the chylomicron carotenoid response [40], the power analysis showed that a group size of 8 would give a power of 80% to find such a difference ($\alpha = 0.05$). data are expressed as means ± SEMs. Postprandial chylomicron carotenoid and triacylglycerol concentrations are expressed as incremental responses (fasting baseline values being zero) in order to diminish the heterogeneity of the responses. However note that similar statistical differences between young and olds were obtained on non corrected data. The areas under the curves (AUCs) of the postprandial chylomicron responses were calculated by the trapezoidal rule. The effects of meals or groups on triacylglycerol AUCs were estimated by two-way analysis of variance (ANOVA). Carotenoid AUCs obtained in the two subject groups (young and old) were compared using an unpaired Student *t*-test. *P* values < 0.05 were considered significant. The statistical comparisons were performed with STATVIEW software (version 5.0; SAS Institute Inc, Cary, NC).

Results

Study population

There were no significant differences between the groups except for BMI and cholesterol concentrations, which were higher in the old than in the young group. This finding reflects a general trend in the population. Dietary intake data showed significantly higher intakes of α and β-carotene and smaller fat intake, expressed as % of energy intake, in the old compared with the young, indicating that, prior to study entry, the general dietary recommendations of increased fruit and vegetable intake have been followed more closely by the old than by the young subjects.

Effect of the vegetable-rich meals on postprandial chylomicron triacylglycerol response in the young and old subjects

The chylomicron triacylglycerol responses to the different vegetable meals and in the two subject groups are shown in **FIGURE 1**. In the young group chylomicron triacylglycerol concentration was maximum 2 h after meal intake, irrespective of test meal. In the old group it was maximum 3 h after the carrot and the spinach meal, and at 2 h after the tomato meal. There was no significant age effect and meal effect on the chylomicron triacylglycerol response when expressed as AUC, even though the AUCs were 27% higher in the old than in the young and the chylomicron triacylglycerol concentrations were generally higher in the old than in the young.

Effect of the carrot meal on postprandial chylomicron β-carotene, α-carotene, 13 cis-β-carotene and retinyl palmitate responses in the young and old subjects

Ingestion of the carrot purée meal elicited a postprandial increase in chylomicron all-*trans* β-carotene, 13 *cis* β-carotene and α-carotene concentrations, with maximum

concentration at 3 and 4 h for the young and the old group, respectively (**FIGURE 2**). Retinyl palmitate concentration was maximum at 3 h in both groups. The whole β-carotene, α-carotene and retinyl palmitate responses (0-9 h AUCs) were not significantly different between the two groups. Standardisation of these responses for chylomicron triacylglycerols did not reveal any significant difference between the two groups (**TABLE 2**). Finally, there was also no significant difference in the chylomicron retinyl-palmitate/(β-carotene + α-carotene) ratio between the two groups: 7.24 ± 1.83 and 5.75 ± 1.38 for the young and old, respectively (data not shown).

Effect of the tomato meal on postprandial chylomicron lycopene response in the young and old subjects

The appearance of lycopene in the chylomicron fraction after ingestion of the tomato meal is shown in **FIGURE 3**. Chylomicron lycopene concentrations increased rapidly to reach a maximum at 3 h in the two groups. The lycopene AUC was not significantly different between the two groups. However, standardisation of the chylomicron lycopene responses for the chylomicron triacylglycerol responses (TABLE 2), revealed a significantly lower response in the old than in the young subjects (- 41%). Note that this difference was also found when postprandial chylomicron lycopene concentrations were not corrected for the concentration at t0 (see the material and methods section).

Effect of the spinach meal on the postprandial chylomicron lutein responses in the young and old subjects.

The chylomicron lutein response to the spinach meal is shown in **FIGURE 4**. This response was markedly different from that of the other carotenoids as it increased with no clear peak during the 9-h postprandial period. Although no AUC can be calculated it was clear that the curves were not markedly different between the two groups.

Evolution of the cis-trans ratios of β-carotene during digestion

The *cis-trans* ratios of β-carotene was no significantly different in the carrot meal (9.5 ± 0.3 % (relative to *cis* + *trans* β-carotene)) and in the chylomicrons collected in the two groups (10.4 ± 2.1 % and 5.6 ± 3.3 % for the Young and the Old group, respectively).

Discussion

The two groups of subjects has been selected to be as much as possible representative of their age class on the basis of plasma lipid parameters. Thus, the subjects of the two age-groups were not compulsorily matched for fasting cholesterol. Indeed, old subjects with young-like values would not have been representative of the elderly population. In fact, Schaefer et al. [41] reported mean cholesterol concentrations of 4.86 mmol/L and 5.87 mmol/L in 20- to 29-year-old men and 60- to 69-year-old men, respectively, indicating that the values found in our groups were representative of these two age classes.

The main objective of this study was to assess whether healthy old persons have impaired carotenoid bioavailability. Since newly absorbed carotenoids are transported by chylomicrons we classically compared the chylomicron carotenoid responses in a group of young and a group of old subjects. This approach has already been used to compare the bioavailability of other fat soluble nutrients, i.e. retinyl palmitate and α-tocopherol, in young and old subjects [39, 42], and was used recently to study the bioavailability of β-carotene [43] and other carotenoids [40]. The main drawback of this approach is that a different chylomicron metabolism, either in the secretion rate of chylomicrons in the plasma or in their clearance rate from the plasma, between the two groups can falsely suggest different carotenoid absorption efficiencies. The higher (+ 27%) chylomicron triacylglycerol response observed in the old group, compared with the young group, agrees with previous observations [42, 44-46]. It has been attributed to a delayed chylomicron clearance in elderly persons due to a lower lipoprotein lipase activity [32, 34, 47]. Although this difference was not significant, it could have led to a misinterpretation of carotenoid data. Accordingly, we systematically standardized the chylomicron carotenoid responses for the chylomicron triacylglycerol responses before drawing any conclusion on the effect of age on carotenoid absorption.

The first striking observation of this study was the chylomicron lutein response to the spinach meal. This response did not exhibit the typical concentration time curve of a rapid increase followed by an exponential decrease in chylomicron concentrations of triacylglycerols or other carotenoids. Several explanation can be proposed. As suggested previously [48] it is possible that a fraction of lutein, which is more polar than β-carotene and lycopene, was absorbed through the portal way. It is also possible that a significant fraction of lutein was metabolized as described previously [49]. Finally it is possible that the gastric emptying rate of the spinach fragments was slower than that of the other vegetables. The spinach was given chopped, whereas the tomatoes and carrots were pureed, and it is well known that only particles less than 1-mm in diameter can pass through the pylorus [50]. Furthermore, in order to provide the same amount of the main carotenoid in the different meals, i.e. 30 mg, a greater amount of spinach was given to the subjects: 260 g as compared to 188 g and 61 g for the carrot purée and the tomato purée, respectively. It is therefore likely that the gastric emptying rate of spinach was slower than that of the other vegetables, leading to a delayed absorption of lutein. This hypothesis is supported by the fact that the chylomicron β-carotene response to the spinach meal did not exhibit a bell-shaped curve but displayed a similar curve to that of lutein (data not shown).

To assess whether carotenoid bioavailability is affected in healthy old we have used the validated chylomicron-response method [40, 43]. The data showed that the chylomicron responses in β-carotene, α-carotene, lycopene and lutein, were not significantly different in the old and in the young groups. Such an observation strongly suggests that there is no major modification of the bioavailability of these carotenoids in healthy old people.

Standardization of the chylomicron carotenoid responses for chylomicron triacylglycerol responses (see above for the reason of this standardization) gave the same results except for lycopene. In fact the significantly lower triacylglycerol-standardized lycopene response in the old group suggests that the bioavailability of this carotenoids is

impaired in these subjects. It is reasonable to suggest that the absorption of lycopene was diminished in old subjects but this effect was partly masked in the non standardized curves by the higher chylomicron response due to the well know impaired chylomicron clearance in these subjects. This effect does not appear to be due to an effect of the tomato matrix on carotenoid bioavailability, as the standardized β-carotene response to the tomato meal was not significantly different between the two groups (data not shown). We therefore suggest that the absorption of this carotenoid is more sensitive to age-related modifications of the physico-chemical conditions prevailing in the gastrointestinal tract [27, 28], given the fact that the absorption efficiency of this carotenoid is very low [15, 51]. This result elegantly explains the results of three studies which showed that fasting plasma lycopene was lower in old subjects than in young ones, whereas the plasma status of other carotenoids was not different [17-19].

Because the *cis* isomers of β-carotene are thought to possess isomer-specific functions [52, 53], we investigated whether the bioavailability of β-carotene isomers and their possible *in vivo cis-trans* isomerization [31] was affected in old subjects. The fact that the proportions of *cis*-isomers of β-carotene in the chylomicrons were not markedly different between the young and the old groups, suggests that this is not the case.

The conversion of provitamin A carotenoids into vitamin A in the intestine is an important vitamin A producing pathway [54]. To our knowledge, human data on the effect of age on this process is lacking. Our results, showing that the chylomicron retinyl-palmitate/(β-carotene + α carotene) ratio was not different in aged subjects compared with young ones, suggest that this process is not markedly affected in healthy old subjects. This result has practical consequences in dietary recommendations for old people, in that there is no evidence to suggest that healthy old cannot obtain the same fraction of their vitamin A from vegetable foods as young people.

In conclusion, these results show that the absorption and the postprandial metabolism of α-carotene, β-carotene and probably lutein is not markedly affected in healthy old persons.

Conversely they suggest that the bioavailability of lycopene is impaired in these subjects, explaining the lower plasma lycopene status observed in elderly subjects in previous studies [17-19]. They also show that there is no major difference in the extent of *cis-trans* isomerization of β-carotene in young and old subjects. Lastly, they show that the intestinal conversion of the provitamin A activity present in carrots, derived from β-carotene and α-carotene, may not be markedly affected in healthy old persons.

Acknowledgments

The authors thank L. Morin for her help in blood collection and M. Brandolini for the analysis of the diet diaries. They also thank Mike Scotter (Central Science Laboratory, York, UK) and Patrick Brachet (INRA, Clermont-Ferrand, France) for having criticised the manuscript. Finally they thank Véronique Braesco (INRA, Clermont-Ferrand, France) for having initiated this European project and for its initial coordination.

TABLE 1

Subject characteristics at the beginning of the study

	Young (n=8)	Old (n=8)	Young *vs* Old [1]
Age (y)	26.8 (3.6)[2]	67.6 (4.2)	$P < 0.001$
BMI (kg/m^2)	22.8 (2.2)	26.4 (2.2)	$P = 0.006$
Glucose (mmol/L)	4.6 (0.4)	5.0 (0.4)	NS
Triacylglycerols (mmol/L)	0.8 (0.3)	1.0 (0.3)	NS
Cholesterol (mmol/L)	4.6 (0.6)	5.8 (0.6)	$P = 0.002$
Daily nutrient intake[3]			
Energy (MJ/d)	10.6 (0.9)	9.4 (1.9)	NS
Carbohydrate (% of energy)	42.4 (6.7)	46.6 (6.2)	NS
Protein (% of energy)	15.9 (2.7)	16.7 (1.9)	NS
Fat (% of energy)	38.8 (4.0)	34.0 (3.6)	$P = 0.019$
Daily micronutrient intake (mg/d)			
Vitamin A	0.7 (0.7)	1.1 (1.3)	NS
beta-carotene	1.9 (1.9)	4.5 (2.5)	$P = 0.033$
alpha-carotene	0.4 (0.4)	1.6 (1.2)	$P = 0.022$
Lycopene	2.6 (1.1)	2.3 (2.1)	NS
Lutein-zeaxanthin	1.0 (1.1)	1.4 (1.0)	NS

[1] Significant differences between the young and old groups as determined by unpaired Student's t-test. NS, not significant. (continued)

(TABLE 1 continued)

[2] Values are means (SD).

[3] Estimated with use of a 5-d food diary at the beginning of the study.

TABLE 2

Chylomicron carotenoid and vitamin A responses (AUCs) standardized for chylomicron triacylglycerol responses (µmol/mmol).

	Young n=8	Old n=8	Young *vs* Old[1]
All-*trans* beta-carotene[2]	22.1 (14.9)[3]	25.9 (20.0)	*P* = 0.68
13-*cis* beta-carotene	2.8 (3.3)	1.2 (1.9)	*P* = 0.25
alpha-carotene	16.1 (7.6)	12.3 (4.2)	*P* = 0.27
Retinyl-palmitate	56.4 (33.5)	40.0 (23.5)	*P* = 0.32
lycopene	41.2 (17.9)	24.9 (8.0)	*P* = 0.04

[1] Significant differences between the young and old groups as determined by unpaired Student's t-test.

[2] beta-carotene, alpha-carotene and retinyl palmitate responses measured after the carrot meal. Lycopene responses measured after the tomato meal.

[3] Values are means (SD).

figures

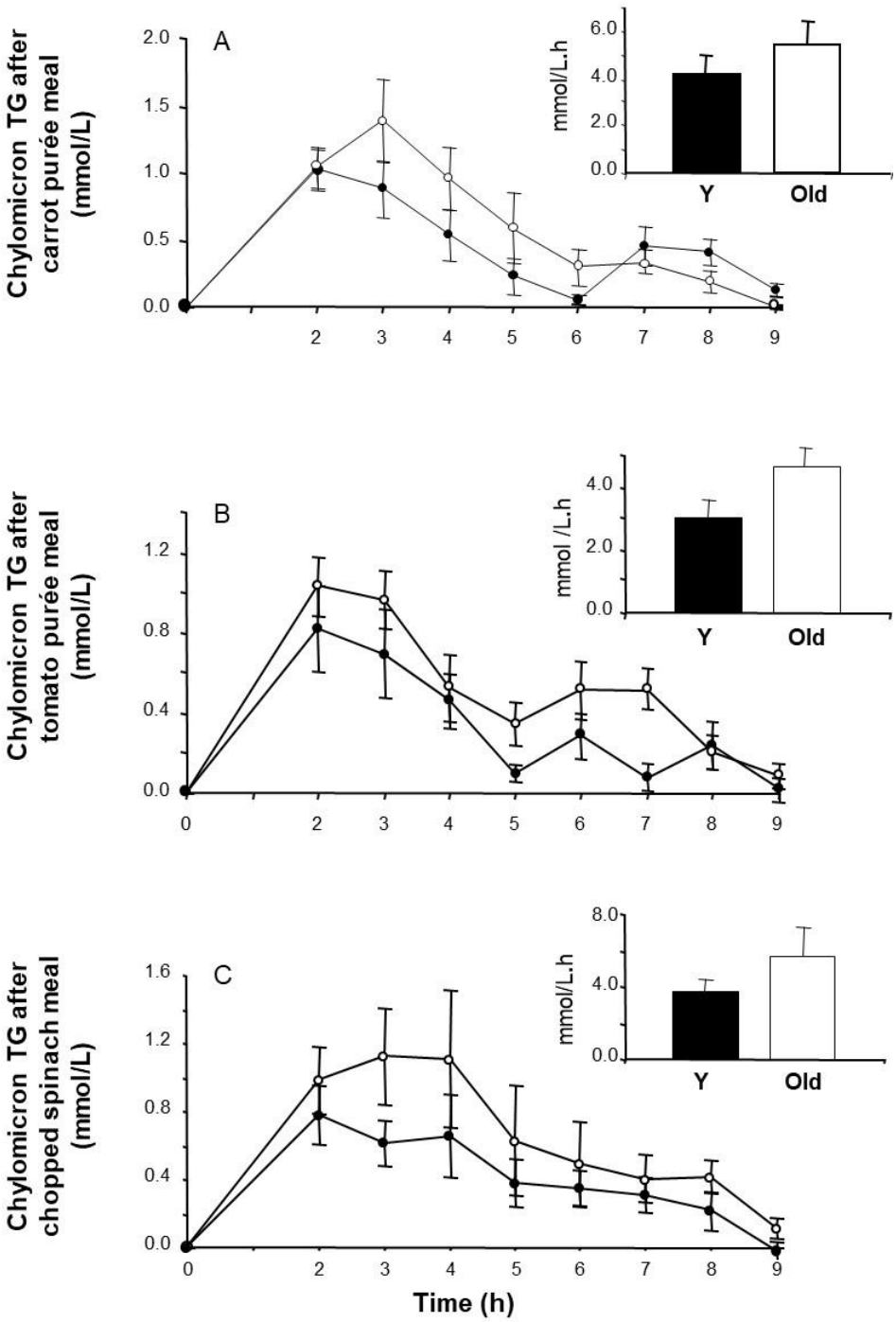
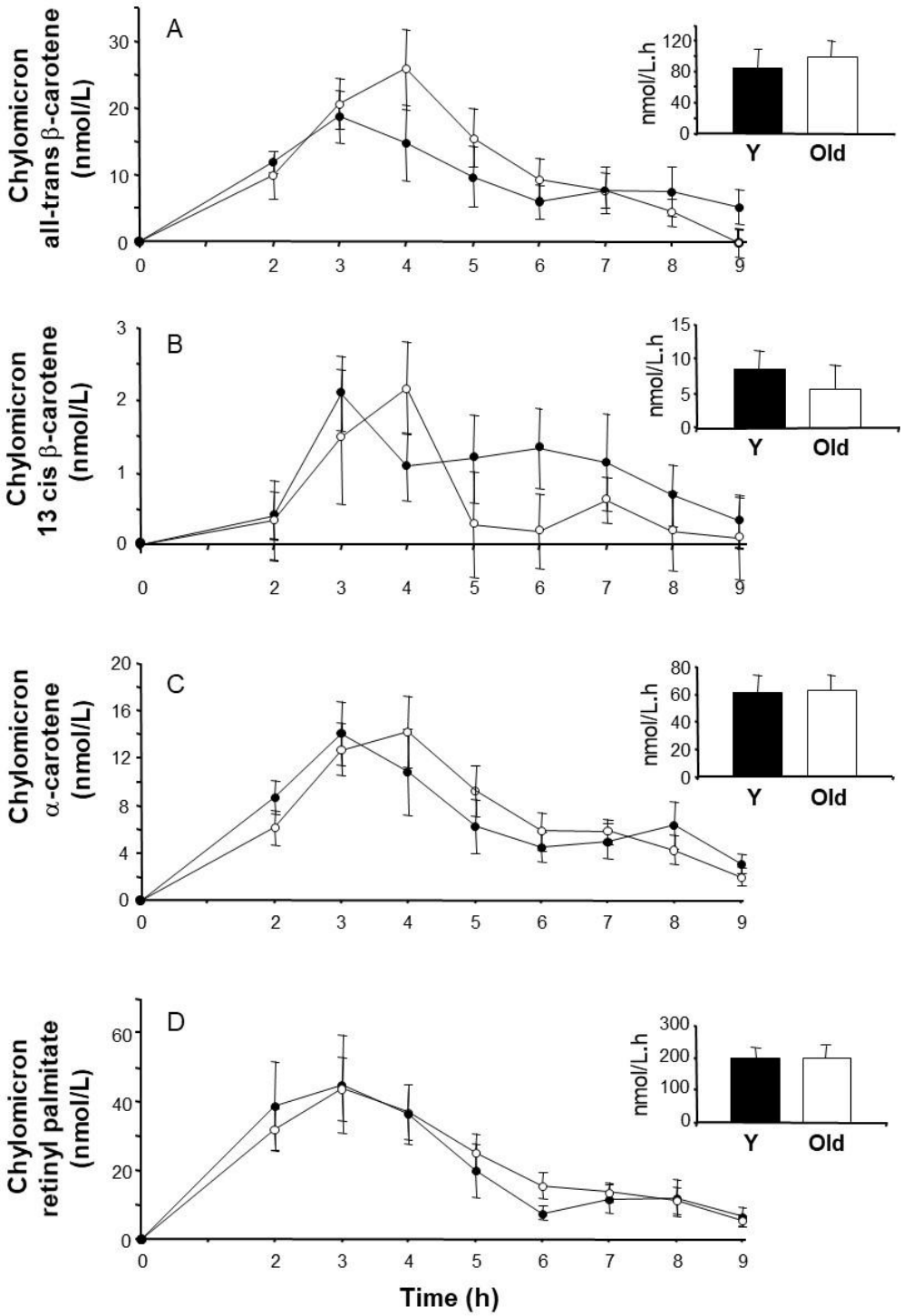


FIGURE 1: Chylomicron triacylglycerol (TG) response to the three different test meals in the young (●) and old (○) volunteers. A: carrot purée meal, B: tomato purée meal, C: chopped spinach meal. Points represent means (SEM) of eight subjects in each group. Insets: Mean (SEM) AUCs of the chylomicron triacylglycerol responses. Y: young, Old: old. Two-factor ANOVA gives no significant meal effect and no age effect on this response.



1 **FIGURE 2**: Mean (SEM) postprandial changes in beta-carotene (A), 13 *cis* beta-carotene
2 (B), alpha-carotene (C) and retinyl-palmitate (D) in the chylomicron fraction after the
3 ingestion of a meal containing carrot purée in the young (●) and old (○) volunteers, n=8 in
4 each group. Insets: Mean (SEM) AUCs of the chylomicron carotenoid and retinyl palmitate
5 responses. There was no significant difference between the AUCs obtained in the two groups
6 (unpaired Student's t test).

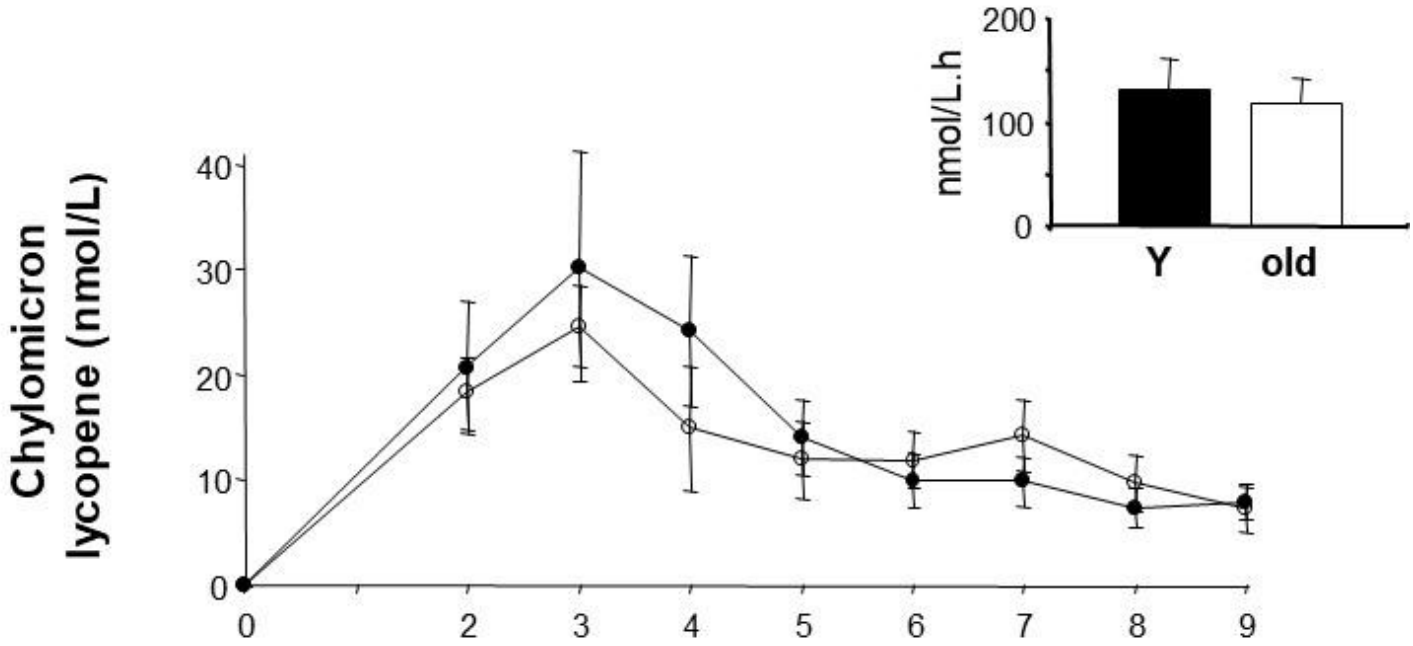


FIGURE 3: Mean (SEM) postprandial changes in lycopene in the chylomicron fraction after the ingestion of a meal providing tomato purée in young (●) and old (○) volunteers, n=8 in each group. Insets: Mean (SEM) AUCs of the chylomicron lycopene response. There was no significant difference between the AUC obtained in the two groups (unpaired Student's t test).

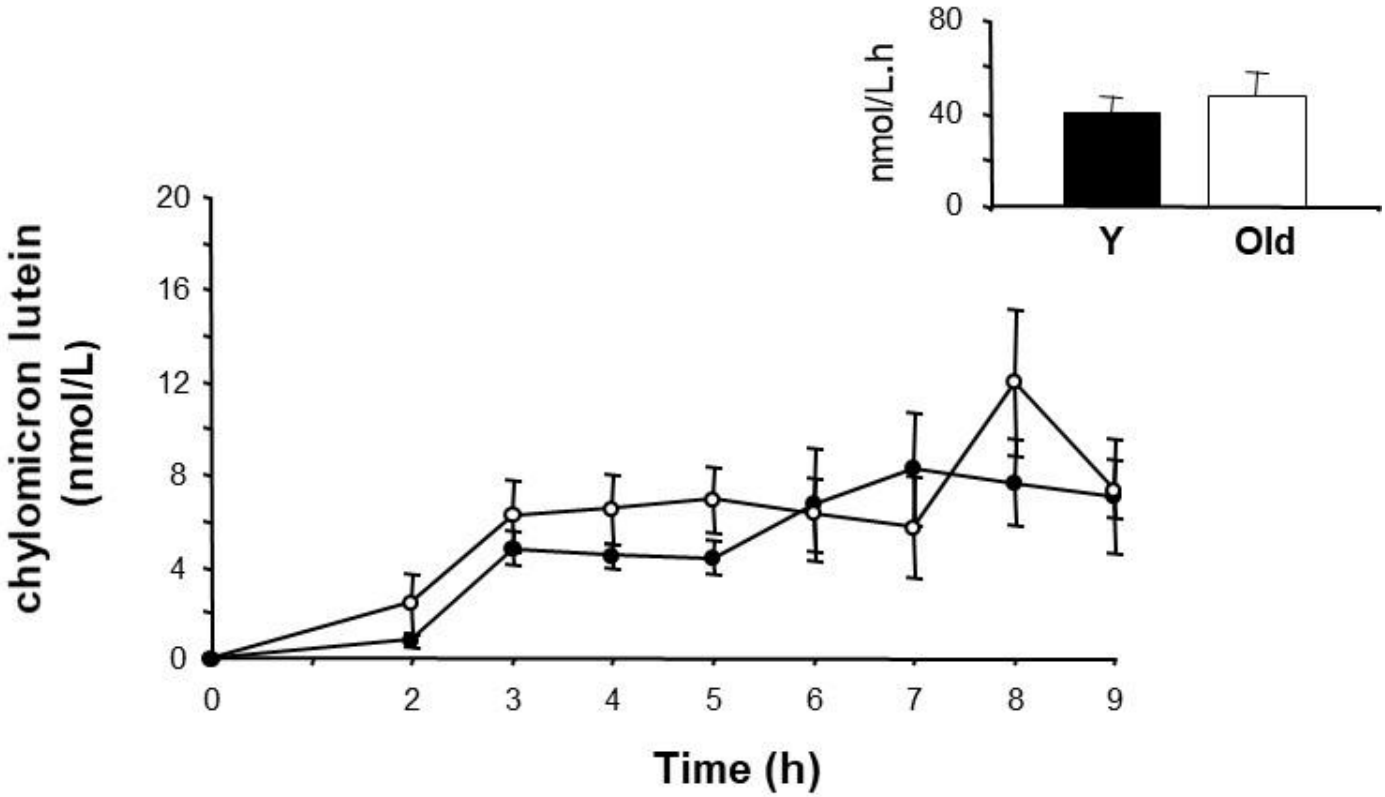


FIGURE 4: Mean (SEM) postprandial changes in lutein in the chylomicron fraction after the ingestion of a meal containing chopped spinach in the young (●) and old (○) volunteers, n=8 in each group.

References

1. Lampe JW (1999) Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr 70: 475S-490S.
2. Broekmans WMR, Klopping-Ketelaars IAA, Schuurman CRWC, Verhagen H, van den Berg H, Kok FJ, vanPoppel G (2000) Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 130: 1578-1583.
3. Krinsky NI (1992) Mechanism of action of biological antioxidants. Proc Soc Exp Biol Med 200: 248-254.
4. Rock CL (1997) Carotenoids: biology and treatment. Pharmacol Ther 75: 185-197.
5. Ziegler RG, Colavito EA, Hartge P, McAdams MJ, Schoenberg JB, Mason TJ, Fraumeni JF (1996) Importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer. J Natl Cancer I 88: 612-615.
6. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Speizer FE, Willett WC, Giovannucci E (2000) Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. Am J Clin Nutr 72: 990-997.
7. Gaziano JM, Manson JE, Branch LG, Colditz GA, Willett WC, Buring JE (1995) A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. Ann Epidemiol 5: 255-260.
8. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merz CN, Fogelman AM (2001) Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. Circulation 103: 2922-2927.

9. Snodderly DM (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 62: 1448S-1461S.

10. Landrum JT, Bone RA (2001) Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 385: 28-40.

11. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S (1996) Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease. New Engl J Med 334: 1150-1155.

12. The Alpha-Tocopherol BCcPSG (1994) The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. New Engl J Med 330: 1029-1035.

13. Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. J Natl Cancer I 91: 317-331.

14. van het Hof KH, Brouwer IA, West CE, Haddeman E, Steegers-Theunissen RP, van Dusseldorp M, Weststrate JA, Eskes TK, Hautvast JG (1999) Bioavailability of lutein from vegetables is 5 times higher than that of beta-carotene. Am J Clin Nutr 70: 261-268.

15. Tyssandier V, Lyan B, Borel P (2001) Main factors governing the transfer of carotenoids from emulsion lipid droplets to micelles. Biochim Biophys Acta 1533: 285-292.

16. Tyssandier V, Choubert G, Grolier P, Borel P (2002) Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins. Int J Vitam Nutr Res 72: 300-308.

17. Vogel S, Contois JH, Tucker KL, Wilson PWF, Schaefer EJ, Lammi-Keefe CJ (1997) Plasma retinol and plasma and lipoprotein tocopherol and carotenoid concentrations in

healthy elderly participants of the Framingham Heart Study. Am J Clin Nutr 66: 950-958.

18. Brady WE, Maresperlman JA, Bowen P, Stacewiczsapuntzakis M (1996) Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J Nutr 126: 129-137.

19. Buiatti E, Munoz N, Kato I, Vivas J, Muggli R, Plummer M, Benz M, Franceschi S, Oliver W (1996) Determinants of plasma anti-oxidant vitamin levels in a population at high risk for stomach cancer. International Journal of Cancer 65: 317-322.

20. Tang GW, Serfaty-Lacrosniere C, Camilo ME, Russell RM (1996) Gastric acidity influences the blood response to a beta-carotene dose in humans. Am J Clin Nutr 64: 622-626.

21. Solomons NW (1996) Aging, gastric acid secretion, and carotene status. Am J Clin Nutr 64: 648-649.

22. Russell RM (2001) Factors in aging that effect the bioavailability of nutrients. J Nutr 131: 1359S-1361S.

23. Maiani G, Mobarhan S, Ceccanti M, Ranaldi L, Gettner S, Bowen P, Friedman H, De Lorenzo A, Ferroluzzi A (1989) Beta-carotene serum response in young and elderly females. Eur J Clin Nutr 43: 749-761.

24. Sugerman SB, Mobarhan S, Bowen P, Sapuntzakis MS, Landenberg P, Henderson C, Kiani R, Friedman H, Lucchesi DJ (1991) Serum time curve characteristics of a fixed dose of B-carotene in young and old men. J Am Coll Nutr 10: 297-307.

25. Carroll YL, Corridan BM, Morrissey PA (1999) Carotenoids in young and elderly healthy humans: dietary intakes, biochemical status and diet-plasma relationships. Eur J Clin Nutr 53: 644-53.

26. Santos MS, Meydani SN, Leka L, Wu DY, Fotouhi N, Meydani M, Hennekens CH, Gaziano JM (1996) Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. Am J Clin Nutr 64: 772-777.

27. Evans MA, Triggs EJ, Cheung M, Broe GA, Creasey H (1981) Gastric emptying rate in the elderly: implications for drug therapy. J Am Geriatr Soc 29: 201-205.

28. Ikuma M, Hanai H, Kaneko E, Hayashi H, Hoshi T (1996) Effects of aging on the microclimate pH of the rat jejunum. Biochim Biophys Acta 1280: 19-26.

29. Russell RM (2000) The aging process as a modifier of metabolism. Am J Clin Nutr 72: 529S-32S.

30. Olson JA (1989) Provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. J Nutr 119: 105-108.

31. You CS, Parker RS, Goodman KJ, Swanson JE, Corso TN (1996) Evidence of cis-trans isomerization of 9-cis-beta-carotene during absorption in humans. Am J Clin Nutr 64: 177-183.

32. Huttunen JK, Ehnholm C, Kekki M, Nikkila EA (1976) Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridaemia: correlations to sex, age and various parameters of triglyceride metabolism. Clin Sci Mol Med 50: 249-60.

33. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E (1993) The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. J Am Diet Assoc 93: 318-323.

34. Weintraub MS, Eisenberg S, Breslow JL (1987) Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 80: 1571-7.

35. Cara L, Dubois C, Borel P, Armand M, Senft M, Portugal H, Pauli AM, Bernard PM, Lairon D (1992) Effects of oat bran, rice bran, wheat fiber, and wheat germ on postprandial lipemia in healthy adults. Am J Clin Nutr 55: 81-8.

36. Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM, Bernard PM, Latge C, Lafont H, Borel P, Lairon D (1994) Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr 60: 374-82.

37. Guerin M, Egger P, Soudant C, Le Goff W, van Tol A, Dupuis R, Chapman MJ (2002) Dose-dependent action of atorvastatin in type IIB hyperlipidemia: preferential and progressive reduction of atherogenic apoB-containing lipoprotein subclasses (VLDL-2, IDL, small dense LDL) and stimulation of cellular cholesterol efflux. Atherosclerosis 163: 287-96.

38. Steghens JP, Lyan B, Le Moel G, Galabert C, Fayol V, Faure H, Grolier P, Cheribi N, Dubois F, Nabet F (2000) Measurement of carotenoids by high pressure liquid chromatography: from difficulties to solutions. Ann Biol Clin-Paris 58: 327-335.

39. Borel P, Mekki N, Boirie Y, Partier A, Grolier P, Alexandre-Gouabau MC, Beaufrere B, Armand M, Lairon D, Azais-Braesco V (1997) Postprandial chylomicron and plasma vitamin E responses in healthy older subjects compared with younger ones. Eur J Clin Invest 27: 812-821.

40. Tyssandier V, Cardinault N, Caris-Veyrat C, Amiot MJ, Grolier P, Bouteloup C, Azais-Braesco V, Borel P (2002) Vegetable-borne lutein, lycopene, and beta-carotene compete for incorporation into chylomicrons, with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. Am J Clin Nutr 75: 526-34.

41. Schaefer EJ, Lichtenstein AH, Lamon-Fava S, McNamara JR, Ordovas JM (1995) Lipoproteins, nutrition, aging, and atherosclerosis. Am J Clin Nutr 61: 726S-740S.

42. Borel P, Mekki N, Boirie Y, Partier A, Alexandre-Gouabau MC, Grolier P, Beaufrere B, Portugal H, Lairon D, Azais-Braesco V (1998) Comparison of the postprandial plasma vitamin A response in young and older adults. J Gerontol A Biol Sci Med Sci 53: B133-B140.

43. van Vliet T, Schreurs WH, van den Berg H (1995) Intestinal beta-carotene absorption and cleavage in men: response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. Am J Clin Nutr 62: 110-116.

44. Krasinski SD, Cohn JS, Schaefer EJ, Russell RM (1990) Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. J Clin Invest 85: 883-892.

45. Cassader M, Gambino R, Ruiu G, Marena S, Bodoni P, Pagano G (1996) Postprandial triglyceride-rich lipoprotein changes in elderly and young subjects. Aging (Milano) 8: 421-8.

46. Relas H, Gylling H, Rajaratnam RA, Miettinen TA (2000) Postprandial retinyl palmitate and squalene metabolism is age dependent. J Gerontol A Biol Sci Med Sci 55: B515-B521.

47. Krauss RM, Levy RI, Fredrickson DS (1974) Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. J Clin Invest 54: 1107-24.

48. van den Berg H, van Vliet T (1998) Effect of simultaneous, single oral doses of beta-carotene with lutein or lycopene on the beta-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction of men. Am J Clin Nutr 68: 82-89.

49. Khachik F, Beecher GR, Smith JC (1995) Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. Journal of Cellular Biochemistry 236-246.

50. Meyer JH, Ohashi H, Jehn D, Thomson JB (1981) Size of liver particles emptied from the human stomach. Gastroenterology 80: 1489-96.

51. Tyssandier V, Borel P, Choubert G, Grolier P, Alexandre-Gouabau MC, Azais-Braesco V (1998) The bioavailability of carotenoids is positively related to their polarity. Sci aliment 18: 324.

52. Lavy A, Amotz AB, Aviram M (1993) Preferential inhibition of LDL oxidation by the all-trans isomer of beta-carotene in comparison with 9-cis beta-carotene. Eur J Clin Chem Clin Biochem 31: 83-90.

53. Hieber AD, King TJ, Morioka S, Fukushima LH, Franke AA, Bertram JS (2000) Comparative effects of all-trans beta-carotene vs. 9-cis beta-carotene on carcinogen-induced neoplastic transformation and connexin 43 expression in murine 10T1/2 cells and on the differentiation of human keratinocytes. Nutr Cancer 37: 234-44.

54. Olson JA (1997) The conversion of carotene to vitamin A (Thomas Moore, 1930). J Nutr 127: S1036-S1038.