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► To cite this version:

Nicolas Cardinault, Viviane Tyssandier, Pascal Grolier, B.M. Winklhofer-Roob, J. Ribalta, et al.. Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. European Journal of Nutrition, 2003, 42, pp.315-323. 10.1007/s00394-003-0426-2. hal-02672502

HAL Id: hal-02672502

<https://hal.inrae.fr/hal-02672502>

Submitted on 20 Apr 2021

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

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

19

20 Running head: Carotenoid bioavailability in healthy old
21

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

24

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

26 postprandial response

1 **Introduction**

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

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

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

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

1 **Subjects and methods**

2

3 *Study population*

4 Sixteen male, non-obese non-smoking volunteers, with a body mass index between
5 19 and 29 kg/m², were recruited. Eight were young adults (20-35 y) and eight were old adults
6 (60-75 y). The study was approved by the regional committee on human experimentation of
7 the university hospital in Clermont-Ferrand (France). Informed written consent was obtained
8 from each volunteer. The subjects did not take any medication nor dietary supplements either
9 during the month before the study started or during the study period. The subjects were
10 apparently healthy, according to clinical and laboratory examination including fasting plasma
11 lipid and glucose concentrations and disease history. The subjects' usual diet was monitored
12 by means of a 5-d food diary the week before the experiment. This diary was analyzed for
13 nutrient composition using diet analyzer software (GENI; Micro 6, Nancy, France). The
14 database of the software was completed for carotenoids with a carotenoid food-composition
15 database [33]. The volunteers' characteristics are presented in **TABLE 1**.

16

17 *Analysis of vegetable carotenoids*

18 Vegetables were purchased at a local supermarket (Stoc, Clermont-Ferrand, France).
19 They were purchased from the same lot. A procedure to accurately extract carotenoids from
20 vegetables was drawn up after preliminary experiments. Several combination of solvents were
21 tried, the best combination being the one that gave the whitest vegetable matrix after
22 extraction. The procedure, which was performed under yellow light, was as follows: 50-70 mg
23 vegetables were added with 7 mL methanol containing 0.57 % MgCO₃ (Sigma, Saint Louis,
24 USA) and 0.2 µg/mL internal standard (echinenone, Roche Vitamines, France). After
25 homogenization for 30 s with a vortex blender, 7 mL trichloromethane (containing 0.005 %
26 butylated hydroxy toluene as an antioxidant) were added. The sample was homogenised again

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

11

12 *Postprandial experiments*

13 Each subject consumed, in a random order, three different test meals on three different
14 days. Each postprandial experiments were separated by at least two weeks. After the subjects
15 had fasted overnight for 12 h, an antecubital vein was catheterized with an intravenous
16 cannula equipped with disposable obturators (Becton Dickinson, Meylan, France). A baseline
17 fasting blood sample was collected and the subjects ate a test meal within 20 ± 2 minutes. The
18 composition of the meal was: 60 g wheat semolina (cooked and hydrated with 120 mL water),
19 40 g peanut oil, 2 pieces of bread (45 g), 1 cooked egg white (35 g), 1 serving of yogurt (125
20 g) and different amounts of vegetables (as natural carotenoid-rich sources). The vegetables
21 were either: 188 g cooked carrot purée (30 mg β -carotene as the main carotenoid and 20.3 mg
22 α -carotene), or 61 g cooked tomato purée (30 mg lycopene as the main carotenoid and 0.54
23 mg β -carotene), or 260 g cooked chopped spinach (30 mg lutein as the main carotenoid and
24 11.6 mg β -carotene). The amount of carotenoids provided by the meals was higher than the
25 amount usually consumed in western countries, but it can be attained under certain dietary
26 conditions and it was designed to accurately follow the fate of these micronutrients in the

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

9

10 *Chylomicron preparation*

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

21

22 *Analytical determinations*

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

1 quantified by reverse-phase HPLC on a Waters system (Waters SA, Saint-Quentin en
2 Yvelines, France). This system comprised a Waters 660 pump, a Waters 717 plus cooled auto-
3 sampler, and a Waters 996 UV-visible diode-array detector. Carotenoids were separated using
4 two columns set in series [38]: a 150 × 4.6 nm RP C₁₈, 3-μm Nucleosyl (Interchim,
5 Montluçon, France), coupled with a 250 × 4.6 nm RP C₁₈, 5-μm Vydac TP54 (Hesperia, CA,
6 USA), and a Hypersil guard column. The mobile phase was an isocratic acetonitrile:
7 dichloromethane: methanol (containing 50 mmol/L ammonium acetate): water (70:10:15:5, by
8 vol) mixture. Carotenoids were detected at 450 nm and identified by comparison of their
9 retention time and spectral analysis (from 300 to 550 nm) with those of pure (> 95%)
10 standards of the following carotenoids: lutein, echinenone, *all-trans* lycopene, α-carotene, *all-*
11 *trans* β-carotene and *13-cis* β-carotene (Roche Vitamines France, Neuilly-sur-Seine, France).
12 In this system the *cis*-isomers of lycopene were not accurately separated from the *all-trans*
13 isomer, thus all lycopene isomers were quantified together. Quantification was conducted
14 using MILLENIUM 32 Waters software (version 3.05.01). Internal standard allowed to
15 calculate and overall recovery yield 75-100%. All the solvents used for carotenoid analyses
16 were HPLC graded from Carlo Erba (Chaussée de Vexin, France).

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

21

22 *Statistical analysis*

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

1 young and old. Assuming a SD of 40% of the mean value in the chylomicron carotenoid
2 response [40], the power analysis showed that a group size of 8 would give a power of 80% to
3 find such a difference ($\alpha = 0.05$). data are expressed as means \pm SEMs. Postprandial
4 chylomicron carotenoid and triacylglycerol concentrations are expressed as incremental
5 responses (fasting baseline values being zero) in order to diminish the heterogeneity of the
6 responses. However note that similar statistical differences between young and olds were
7 obtained on non corrected data. The areas under the curves (AUCs) of the postprandial
8 chylomicron responses were calculated by the trapezoidal rule. The effects of meals or groups
9 on triacylglycerol AUCs were estimated by two-way analysis of variance (ANOVA).
10 Carotenoid AUCs obtained in the two subject groups (young and old) were compared using an
11 unpaired Student *t*-test. *P* values < 0.05 were considered significant. The statistical
12 comparisons were performed with STATVIEW software (version 5.0; SAS Institute Inc, Cary,
13 NC).
14

1 **Results**

2

3 *Study population*

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

11

12 *Effect of the vegetable-rich meals on postprandial chylomicron triacylglycerol response in the* 13 *young and old subjects*

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

22

23 *Effect of the carrot meal on postprandial chylomicron β -carotene, α -carotene, 13 cis- β -* 24 *carotene and retinyl palmitate responses in the young and old subjects*

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

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

9

10 *Effect of the tomato meal on postprandial chylomicron lycopene response in the young and* 11 *old subjects*

12 The appearance of lycopene in the chylomicron fraction after ingestion of the tomato
13 meal is shown in **FIGURE 3**. Chylomicron lycopene concentrations increased rapidly to reach
14 a maximum at 3 h in the two groups. The lycopene AUC was not significantly different
15 between the two groups. However, standardisation of the chylomicron lycopene responses for
16 the chylomicron triacylglycerol responses (**TABLE 2**), revealed a significantly lower response
17 in the old than in the young subjects (- 41%). Note that this difference was also found when
18 postprandial chylomicron lycopene concentrations were not corrected for the concentration at
19 t_0 (see the material and methods section).

20

21 *Effect of the spinach meal on the postprandial chylomicron lutein responses in the young and* 22 *old subjects.*

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

1

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

3 The *cis-trans* ratios of β -carotene was no significantly different in the carrot meal
4 (9.5 \pm 0.3 % (relative to *cis* + *trans* β -carotene)) and in the chylomicrons collected in the two
5 groups (10.4 \pm 2.1 % and 5.6 \pm 3.3 % for the Young and the Old group, respectively).

6

1 Discussion

2

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

10 The main objective of this study was to assess whether healthy old persons have
11 impaired carotenoid bioavailability. Since newly absorbed carotenoids are transported by
12 chylomicrons we classically compared the chylomicron carotenoid responses in a group of
13 young and a group of old subjects. This approach has already been used to compare the
14 bioavailability of other fat soluble nutrients, i.e. retinyl palmitate and α -tocopherol, in young
15 and old subjects [39, 42], and was used recently to study the bioavailability of β -carotene [43]
16 and other carotenoids [40]. The main drawback of this approach is that a different
17 chylomicron metabolism, either in the secretion rate of chylomicrons in the plasma or in their
18 clearance rate from the plasma, between the two groups can falsely suggest different
19 carotenoid absorption efficiencies. The higher (+ 27%) chylomicron triacylglycerol response
20 observed in the old group, compared with the young group, agrees with previous observations
21 [42, 44-46]. It has been attributed to a delayed chylomicron clearance in elderly persons due to
22 a lower lipoprotein lipase activity [32, 34, 47]. Although this difference was not significant, it
23 could have led to a misinterpretation of carotenoid data. Accordingly, we systematically
24 standardized the chylomicron carotenoid responses for the chylomicron triacylglycerol
25 responses before drawing any conclusion on the effect of age on carotenoid absorption.

1 The first striking observation of this study was the chylomicron lutein response to the
2 spinach meal. This response did not exhibit the typical concentration time curve of a rapid
3 increase followed by an exponential decrease in chylomicron concentrations of
4 triacylglycerols or other carotenoids. Several explanation can be proposed. As suggested
5 previously [48] it is possible that a fraction of lutein, which is more polar than β -carotene and
6 lycopene, was absorbed through the portal way. It is also possible that a significant fraction of
7 lutein was metabolized as described previously [49]. Finally it is possible that the gastric
8 emptying rate of the spinach fragments was slower than that of the other vegetables. The
9 spinach was given chopped, whereas the tomatoes and carrots were pureed, and it is well
10 known that only particles less than 1-mm in diameter can pass through the pylorus [50].
11 Furthermore, in order to provide the same amount of the main carotenoid in the different
12 meals, i.e. 30 mg, a greater amount of spinach was given to the subjects: 260 g as compared to
13 188 g and 61 g for the carrot purée and the tomato purée, respectively. It is therefore likely
14 that the gastric emptying rate of spinach was slower than that of the other vegetables, leading
15 to a delayed absorption of lutein. This hypothesis is supported by the fact that the chylomicron
16 β -carotene response to the spinach meal did not exhibit a bell-shaped curve but displayed a
17 similar curve to that of lutein (data not shown).

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

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

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

12 Because the *cis* isomers of β -carotene are thought to possess isomer-specific functions
13 [52, 53], we investigated whether the bioavailability of β -carotene isomers and their possible
14 *in vivo cis-trans* isomerization [31] was affected in old subjects. The fact that the proportions
15 of *cis*-isomers of β -carotene in the chylomicrons were not markedly different between the
16 young and the old groups, suggests that this is not the case.

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

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

1 Conversely they suggest that the bioavailability of lycopene is impaired in these subjects,
2 explaining the lower plasma lycopene status observed in elderly subjects in previous studies
3 [17-19]. They also show that there is no major difference in the extent of *cis-trans*
4 isomerization of β -carotene in young and old subjects. Lastly, they show that the intestinal
5 conversion of the provitamin A activity present in carrots, derived from β -carotene and α -
6 carotene, may not be markedly affected in healthy old persons.

7

1 **Acknowledgments**

2
3 The authors thank L. Morin for her help in blood collection and M. Brandolini for the analysis of the
4 diet diaries. They also thank Mike Scotter (Central Science Laboratory, York, UK) and Patrick Brachet
5 (INRA, Clermont-Ferrand, France) for having criticised the manuscript. Finally they thank Véronique
6 Braesco (INRA, Clermont-Ferrand, France) for having initiated this European project and for its initial
7 coordination.

8

1 **TABLE 1**2 *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)	<i>P</i> < 0.001
BMI (kg/m ²)	22.8 (2.2)	26.4 (2.2)	<i>P</i> = 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)	<i>P</i> = 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)	<i>P</i> = 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)	<i>P</i> = 0.033
alpha-carotene	0.4 (0.4)	1.6 (1.2)	<i>P</i> = 0.022
Lycopene	2.6 (1.1)	2.3 (2.1)	NS
Lutein-zeaxanthin	1.0 (1.1)	1.4 (1.0)	NS

3

4 ¹ Significant differences between the young and old groups as determined by unpaired

5 Student's t-test. NS, not significant. (continued)

6 (TABLE 1 continued)

7 ² Values are means (SD).8 ³ Estimated with use of a 5-d food diary at the beginning of the study.

1 **TABLE 2**

2 *Chylomicron carotenoid and vitamin A responses (AUCs) standardized for*
 3 *chylomicron triacylglycerol responses ($\mu\text{mol}/\text{mmol}$).*

	Young n=8	Old n=8	Young vs Old ¹
All- <i>trans</i> beta-carotene ²	22.1 (14.9) ³	25.9 (20.0)	$P = 0.68$
13- <i>cis</i> 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)	$P = 0.04$

4

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

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

9 ³ Values are means (SD).

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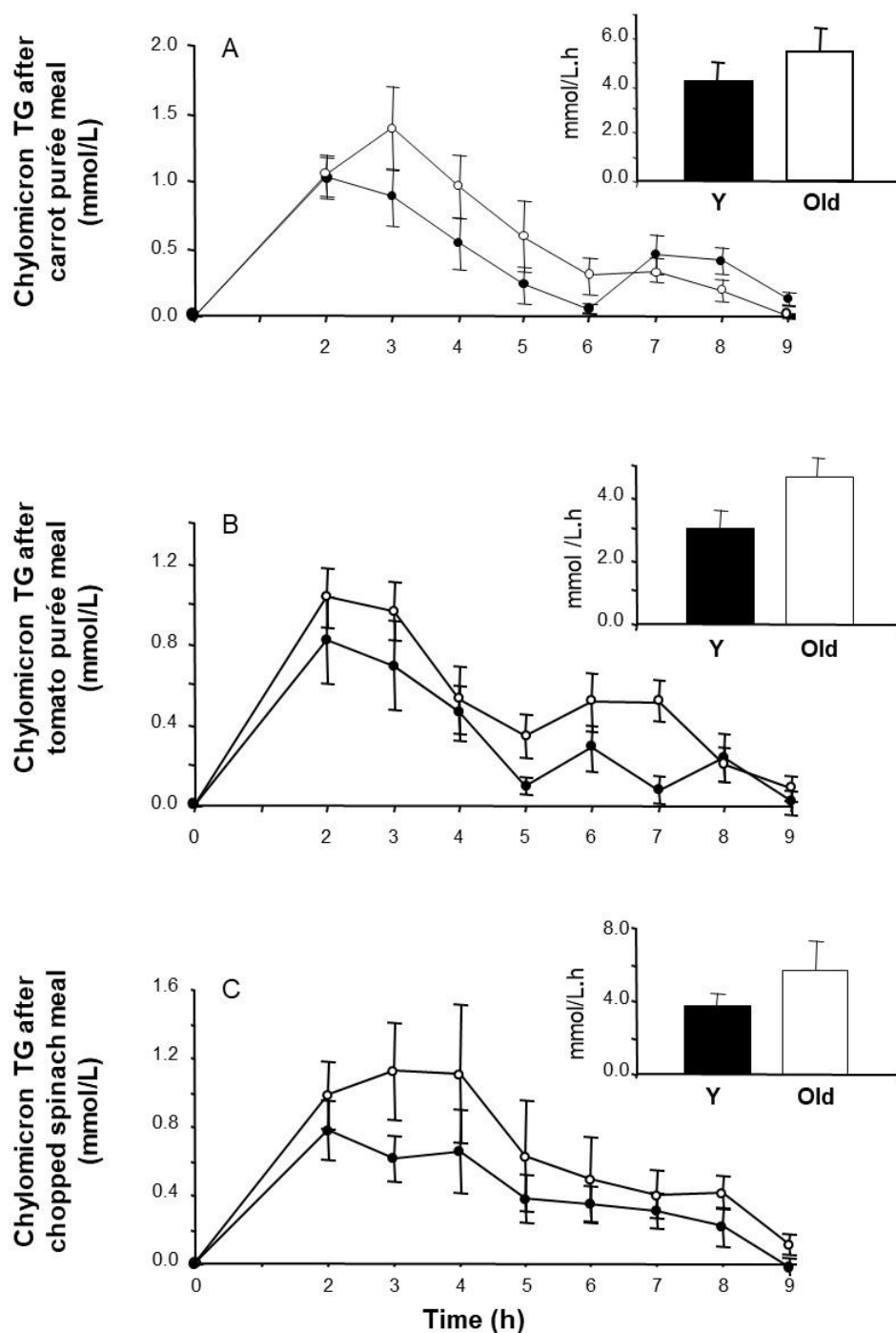
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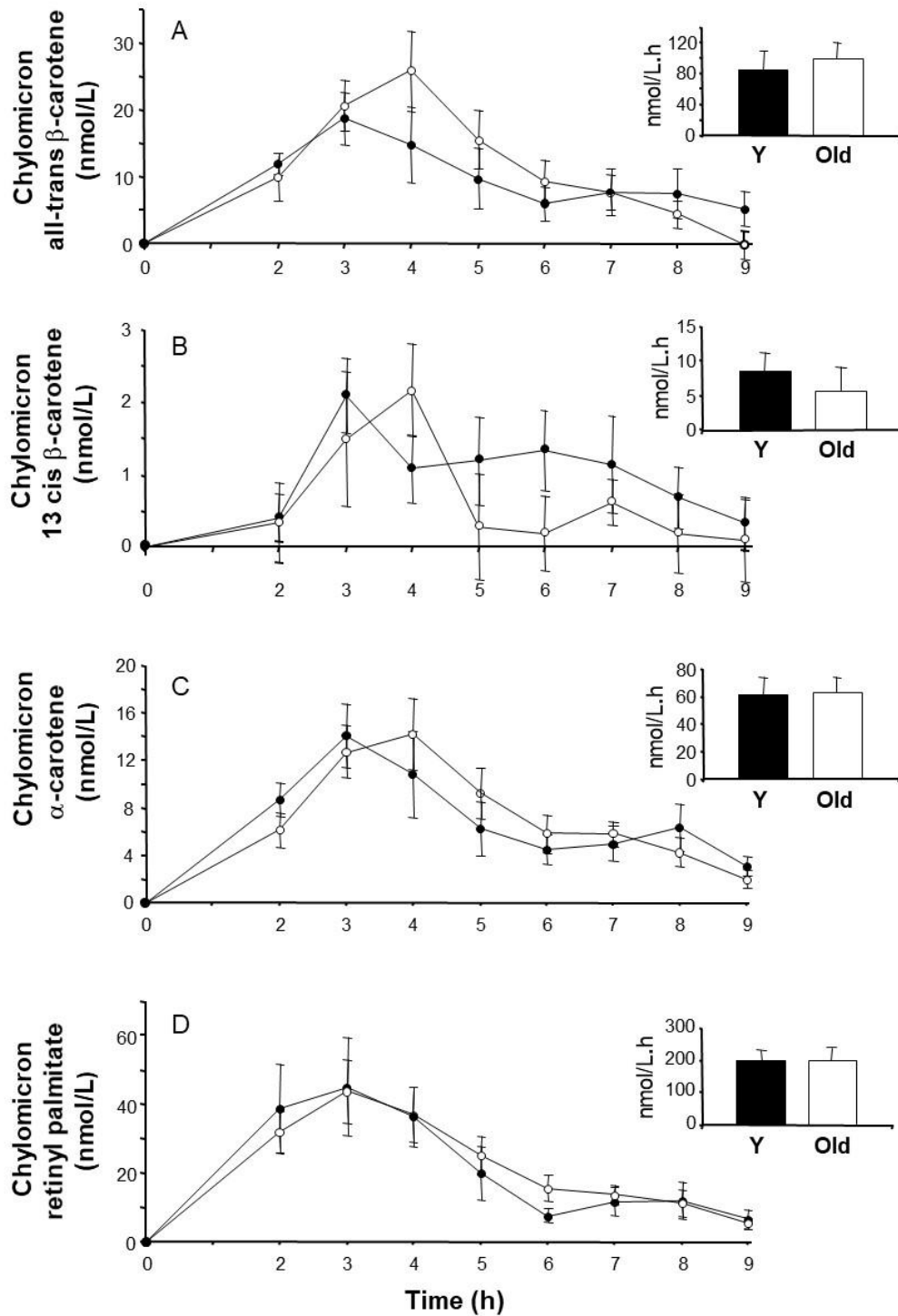
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1 figures

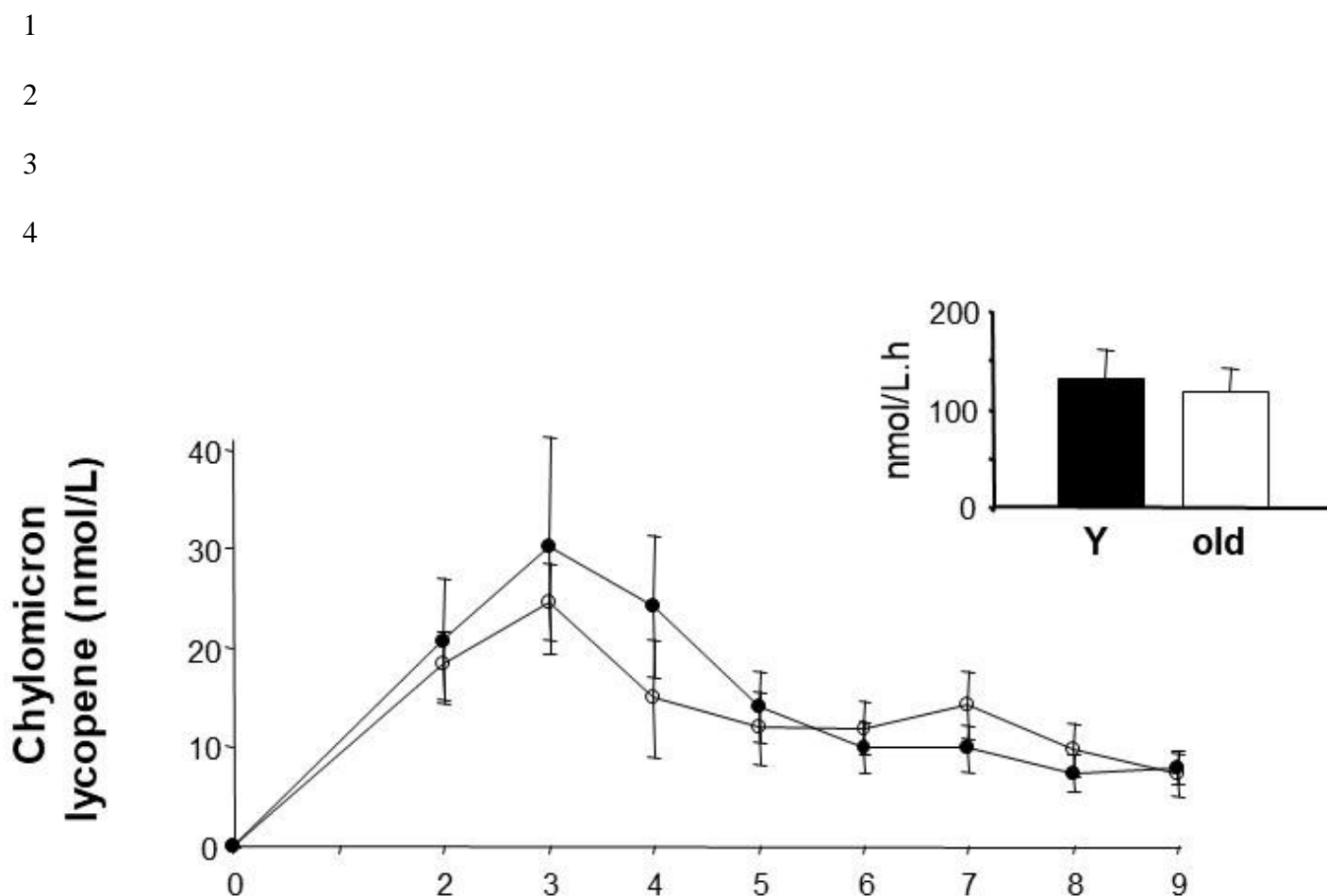


2

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



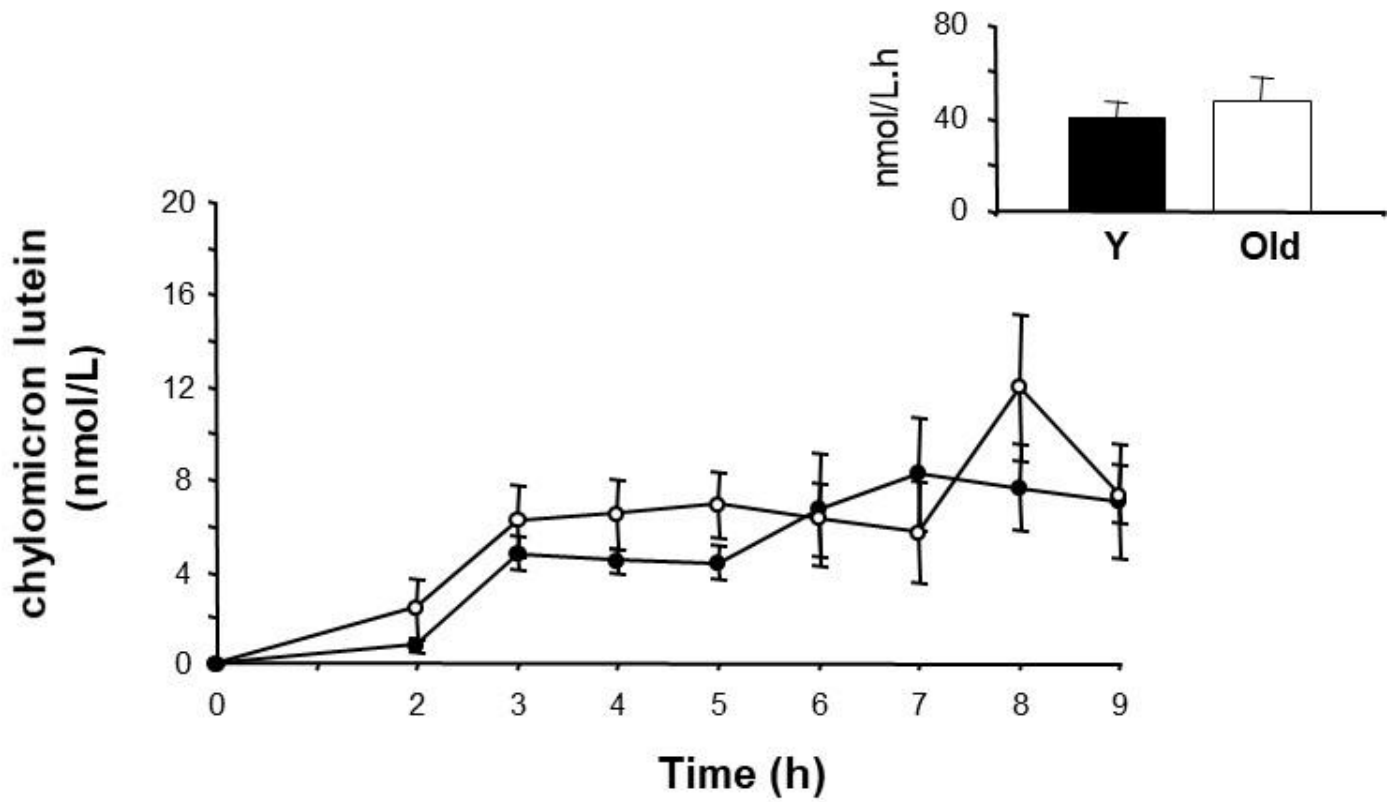
1 **FIGURE 2:** Mean (SEM) postprandial changes in beta-carotene (A), 13 *cis* beta-carotene
2 (B), alpha-carotene (C) and retinyl-palmitate (D) in the chylomicron fraction after the
3 ingestion of a meal containing carrot purée in the young (●) and old (○) volunteers, n=8 in
4 each group. Insets: Mean (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).



5
6
7 **FIGURE 3:** Mean (SEM) postprandial changes in lycopene in the chylomicron fraction
8 after the ingestion of a meal providing tomato purée in young (●) and old (○) volunteers, n=8
9 in each group. Inset: Mean (SEM) AUCs of the chylomicron lycopene response. There was
10 no significant difference between the AUC obtained in the two groups (unpaired Student's t
11 test).

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

8

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2

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