Comparison of the bioavailability and intestinal absorption sites of phytoene, phytofluene, lycopene and β-carotene

Paula Mapelli-Brahm[2], Marielle Margier[1], Charles Desmarchelier[1], Charlotte Halimi[1], Marion Nowicki[1], Patrick Borel[1], Antonio J. Meléndez-Martínez[2] and Emmanuelle Reboul[1]*

[1] Aix Marseille Univ, INRA, INSERM, C2VN, Marseille, France.

[2] Food Colour & Quality Lab, Area of Nutrition & Food Science, Universidad de Sevilla, 41012, Seville, Spain.

***Address correspondence to**: Emmanuelle Reboul, Center for CardioVascular and Nutrition research (C2VN), UMR 1263 INSERM/1260 INRA/Aix-Marseille Université, Faculté de Médecine la Timone, 27 boulevard Jean-Moulin, 13385 Marseille Cedex 5 FRANCE. Tel: (+33).4.91.32.42.78; E-mail: Emmanuelle.Reboul@univ-amu.fr

Abbreviated running Title: Bioavailability and intestinal absorption sites of tomato carotenoids

Abstract

The mechanisms of main tomato carotenes (phytoene, phytofluene, lycopene and β-carotene) intestinal absorption are still only partly understood. We thus compared carotene bioavailability in mice after gavage with carotene-rich oil-in-water emulsions. We also determined each carotene absorption profile along the duodenal-ileal axis of the intestine to identify their respective absorption sites and compared these profiles with the gene expression sites of their identified transporters, i.e. SR-BI and CD36. Our data show that phytofluene presented a significantly higher bioavailability compared to lycopene and β-carotene (areas under the curve of 0.76±0.09 vs. 0.30±0.05, 0.09±0.05 and 0.08±0.01 µmol/L.h for phytofluene, phytoene, lycopene and β-carotene, respectively). β-carotene was mostly converted in the proximal and median intestine. Phytoene and phytofluene accumulation tended to be more important in the distal intestine, which did not correlate with the proximal expression of both *Scarb1* and *CD36*. Overall, these results highlight the high bioavailability of phytofluene.

Key words: tomato carotenoid, digestion, enterocyte, intestine, mice.

Abbreviations: CD36 (CD36 molecule), CRBPII (Cytosolic Retinol Binding Protein Type II), LRAT (Lecithin-Retinol Acyltransferase), SR-BI (Scavenger Receptor class B type I), NPC1-L1 (NPC1 like intracellular cholesterol transporter 1).

1. Introduction

Carotenoids represent a broad family of about 800 molecules synthetized by plants and microorganisms, around 40 of which are found in significant amounts in human diets. Non-oxygenated and oxygenated carotenoids belong to the carotene and xanthophyll family, respectively. The main dietary carotenes are β- and α-carotene, and lycopene. Interestingly, a recent emphasis has been made on two other carotenes: phytoene and phytofluene (see for review: (Reboul, 2019)). These molecules can be found in high (0.5–2 mg/100 g of fresh weight) or very high (> 2 mg/100 g of fresh weight) concentrations (George Britton & Khachik, 2009) in tomatoes, carrots, oranges and apricots, and are detectable in significant amounts in both human plasma and tissues, where they might display specific health benefits (Melendez-Martinez, Mapelli-Brahm, Benitez-Gonzalez, & Stinco, 2015). Indeed, phytoene and phytofluene present particular properties due to their molecular structures. While other dietary carotenoids contain at least 10 conjugated double bounds, phytoene and phytofluene only present 3 and 5 conjugated double bounds, respectively (Table 1). These reduced numbers of double bounds are responsible for their lack of color, their twisted shape, and likely affect both their bioavailability and functional properties (Melendez-Martinez, Mapelli-Brahm, Benitez-Gonzalez, & Stinco, 2015).

Both phytoene and phytofluene were shown to display a higher bioaccessibility, i.e a better incorporation into mixed micelles, compared to lutein, β-carotene and lycopene (Jeffery, Turner, & King, 2012; Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Phytofluene was also shown to be more absorbed by human intestinal cells in culture compared to phytoene (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Finally, phytoene was more bioavailable than lycopene in Mongolian gerbils

(Moran, Clinton, & Erdman, 2013) and in humans (Moran, Novotny, Cichon, Riedl, Rogers, Grainger, et al., 2016). However, no comprehensive comparison of the bioavailability of the 4 tomato carotenes, i.e. phytoene, phytofluene, lycopene and β-carotene, has been conducted so far *in vivo*.

To gain insight into carotene intestinal absorption, we thus i) compared the postprandial responses of phytoene, phytofluene, lycopene and β-carotene after gavage in mice and ii) investigated their absorption sites along mouse intestine.

2. Materials and methods

2.1 Chemicals

All-E-β-carotene (>97% pure) was purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). *All-E*-lycopene (>95% pure) and *all-E*-echinenone (>95% pure) were generous gifts from DSM Nutritional Products Ltd (Basel, Switzerland). Phytoene (>99% pure, mainly present as *Z*-phytoene) and phytofluene (> 99% pure, mainly present as *Z*-phytofluene) were isolated from tomato extract as described previously (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017).

2.2 Preparation of carotene-enriched emulsions for mouse experiments

To deliver carotenes to mice, carotene-rich emulsions were prepared as previously described (Reboul, Goncalves, Comera, Bott, Nowicki, Landrier, et al., 2011) with minor modifications. An appropriate volume of the stock solution of each carotenoid was transferred to Eppendorf tubes to obtain a final amount of 2 mg in each tube. Stock solution solvent was evaporated under nitrogen. To facilitate the subsequent solubilisation of carotenoids in triolein, 15 µL of hexane were added. The sample was vortexed 20 sec and then 1 mL of triolein were added. The mixture was vortexed 1 min and the hexane was evaporated under nitrogen. After 1 min of vortex, 1 mL of an aqueous solution (NaCl 0.9%, BSA 2% and phospholipids 0.1%) were added. The sample was vortexed for 6 min and then probe-sonicated (20 sec two times) to obtain homogenous and stable emulsions that were used within 10 min. Mice were force-fed with 300 µL of emulsions containing 250 µg of either lycopene, phytoene, phytofluene or β-carotene.

2.3 Animals and sample collection

Six-week-old wild-type male C57BL/6 Rj mice (21-23 g) were purchased from Janvier (Janvier, Le-Genest-St-Isle, France). The mice were housed in a temperature-, humidity- and light-controlled room. They were given a standard chow diet and water *ad libitum*. Mice were fasted overnight before each experiment. The protocol was approved by the ethics committee of Marseille (agreement APAFIS#13473-20180209184003330 v3).

One week before the experiment, a blood sample (≈ 50 µL) was collected from the submandibular vein at fast to evaluate mouse carotenoid status. On the day of the experiment, the mice (n= 4 to 6) were force-fed with one of the carotene-rich emulsions and additional blood samples (≈ 50 µL) were taken 1.5 h, 3h and 4.5 h after gavage. After 6 h, a last blood sample was taken by intracardiac puncture under sevoflurane anesthesia. The plasma was separated from the blood by centrifugation by using 3.8% sodium citrate as an anticoagulant. The intestine of each animal was then quickly harvested after euthanasia by cervical dislocation, and carefully rinsed with ice-cold PBS. The small intestine was cut into 5 equal segments of 6 cm (representing the duodenum, the proximal – medial – distal jejunum and the ileum) along a total length of 30 cm. All samples were suspended in 500 µL PBS, homogenized and rapidly stored at -80 °C until analysis.

2.4 Carotene extraction and HPLC analysis

Carotenes were extracted from blood or intestine samples using the method previously described (Goncalves, Gleize, Roi, Nowicki, Dhaussy, Huertas, et al., 2013; Goncalves, Margier, Roi, Collet, Niot, Goupy, et al., 2014). The internal standard was echinenone. Phytofluene and phytoene were not reported to be metabolized in the intestine (Engelmann, Clinton, & Erdman, 2011) and were thus assayed in their native form. Conversely, as

lycopene can be isomerized in the intestine (Richelle, Sanchez, Tavazzi, Lambelet, Bortlik, & Williamson, 2010), both *Z*- and *all-E*-lycopene were quantified and results were pooled. Similarly, as β-carotene is highly converted into retinyl esters in mice (Ribaya-Mercado, Holmgren, Fox, & Russell, 1989), retinyl oleate, linoleate, palmitate and stearate concentrations were measured together with β-carotene in samples obtained after gavage with the β-carotene-rich emulsion. Note that no retinyl esters were detected in the plasma of mice that received nonprovitamin A carotene. Retinol deriving from β-carotene, i.e. [retinol measured in mucosa samples after β-carotene gavage corrected for retinol present in mouse mucosa after nonprovitamin A carotenoid gavage], was also evaluated in mouse intestine. Retinol and retinyl esters recovered in these samples were then expressed in pmol equivalent to β-carotene (1 mol of β-carotene = ½ mol retinol or ½ mol retinyl esters). After lipid extraction with hexane, dried residues were dissolved in 200 µL of mobile phase (20% methyl-*tert*-buthyl ether – 80% methanol). A volume of 180 µL was used for HPLC analysis. The HPLC systems and methods were set up according to previous studies (Gleize, Steib, Andre, & Reboul, 2012; Reboul, Trompier, Moussa, Klein, Landrier, Chimini, et al., 2009). Each carotene was identified by retention time compared with pure standards.

2.5 RNA extraction and transporter gene expression analysis along mouse intestine

Intestines of 3 fasting mice were harvested, carefully rinsed with ice-cold PBS, and cut in 5 segments of equal length. Total RNA was extracted from scraped mucosa (n = 3 per group) using TRIzol reagent (Euromedex, France). Total RNA concentration and quality were measured with the use of the BioDrop µLite (Isogen Life Science, De Meern, The Netherlands). cDNAs were synthesized from 1 µg total RNA. Real time quantitative PCR assays were performed with Sybr green mixes using a thermocycler Light Cycler 480 (Roche). For each condition, expression was quantified in duplicate, and *18s* rRNA was used

as endogenous control in the comparative cycle threshold (CT) method. Primer sequences were *18s* [F: CGCCGCTAGAGGTGAAATTCT and R: CATTCTTGGCAAATGCTTTCG], *Scarb1* [F: AGCGCCAAGGTCATCATC and R: CTGCCTAACATCTTGGTCCTG], *cd36* [F: CTTGTGTTTTGAACATTTCTGCTT and R: TTGTACCTATACTGTGGCTAAATGAGA].

2.6 Calculations and statistical analyses

Carotenoid bioavailability was assessed by measuring the AUC of their postprandial plasma concentrations over 6 h, using the trapezoidal rule.

Results are expressed as means ± SEM. For ANOVA, normality of the residuals was checked by Kolmogorov-Smirnov, using the Lilliefors correction, and Shapiro-Wilk tests while equality of variances was checked by Levene's test. Differences in postprandial carotenoid peak concentrations (C_{max}) and intestinal carotenoid concentrations were analyzed using ANOVA. Tukey's test, which maintains the family-wise error rate at alpha = 0.05, was used as a post hoc for pairwise comparisons. Due to heteroscedasticity not resolved by data transformation, differences in carotenoid bioavailability were analyzed using the Kruskal-Wallis test. Dunn's test, with the Bonferroni adjustment was used as a post hoc test for pairwise comparisons. Values of $p<0.05$ were considered significant. Statistical analyses were performed using SPSS (version 20, SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Postprandial plasma carotene responses in mice

We first assessed the appearance of carotenes in mouse blood compartment after gavage with the different emulsions (Figure 1). β-carotene was largely recovered as retinyl esters (> 95%). Phytoene, lycopene and β-carotene peaked in plasma around 3 h after gavage, while phytofluene peaked around 4.5 h.

The 4 carotenoids elicited significantly ($p<0.001$) different C_{max} (phytofluene: 0.22 ± 0.03^{a}; phytoene: 0.10 ± 0.02^{b}; lycopene: 0.03 ± 0.02^{bc}; β-carotene: 0.02 ± 0.00^{c}). They also displayed significantly ($p=0.002$) different bioavailabilities, with phytofluene exhibiting significantly higher bioavailability compared to lycopene and β-carotene (0.76 ± 0.09^{a}, 0.30 ± 0.06^{ab}, 0.09 ± 0.06^{b} and 0.08 ± 0.01^{b} μmol/L.h for phytofluene, phytoene, lycopene and β-carotene, respectively).

3.2 Carotene accumulation by mouse intestine

The accumulation of the different carotenes did not significantly differ from one segment to another (Figure 2). β-Carotene (i.e. β-carotene + newly-formed retinol + retinyl esters) conversion by intestinal mucosa was preferentially localized in the proximal and median intestine (Figure 2D). Indeed, 90.0 to 92.5% of the β-carotene was converted in the 3 first segments (duodenum, proximal and median jejunum), while 53.2 to 54.4% was converted in the last 2 segments (distal jejunum and ileum). Interestingly, phytoene and phytofluene tended to accumulate in the distal part of the intestine (Figures 2A and 2B, respectively). For

example, the amount of phytoene recovered in the ileum was ≈ 4-fold higher than that found in the duodenum.

Overall, the total quantity of the different carotenes in mouse intestinal mucosa were: 3.13 ± 0.46^{a} nmol for β-carotene (recovered in the form of β-carotene, retinol and retinyl esters), 0.87 ± 0.16^{ab} nmol for phytofluene, 0.48 ± 0.13^{bc} for phytoene and 0.29 ± 0.16^{c} for lycopene (p=0.001).

3.3 Carotene transporter gene expression in mouse intestine

CD36 molecule (CD36) was mainly expressed in the proximal jejunum while Scavenger Receptor Class B type I (SR-BI, encoded by *Scarb1*) was mainly expressed in the duodenum. Both showed a decreasing expression along the duodenal-ileal axis of the intestine (Figure 3).

Discussion

The molecular mechanisms involved in the absorption of carotenoids are only partly understood. Initially, Hollander and colleagues suggested that β-carotene was absorbed by passive diffusion. However, studies have reconsidered this assumption and have shown that carotenoid absorption mechanisms are more complex than previously described (Reboul & Borel, 2011). In particular, we used Caco-2 cells and transfected Griptite cells to show that the enterocyte uptake of provitamin-A carotenoids, including β-carotene, involved both SR-BI and CD36. This was comforted by the fact that genetic variations in the genes encoding these membrane proteins were associated with plasma concentrations of provitamin A carotenoids at a population level (Borel, Lietz, Goncalves, Szabo de Edelenyi, Lecompte, Curtis, et al., 2013). Recently, we also showed that SR-BI, but not CD36, was involved in phytoene and phytofluene uptake in these two cell models (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). NPC1-like intracellular cholesterol transporter 1, NPC1L1, which is another fat-soluble micronutrient transporter (Reboul & Borel, 2011), was neither involved in the transport of lycopene (Moussa, Landrier, Reboul, Ghiringhelli, Comera, Collet, et al., 2008) nor in that of colorless carotenoids (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Overall, SR-BI emerges as an efficient and ubiquitous carotenoid transporter at the intestinal level.

Despite a common SR-BI-dependent absorption pathway, carotene absorption efficiency seems highly variable depending on the molecule considered. We thus performed a comprehensive study to compare the bioavailability of the main tomato carotenes using a postprandial model in mice. This model has previously been used to investigate both carotenoids (Mensi, Borel, Goncalves, Nowicki, Gleize, Roi, et al., 2014) and fat-soluble vitamins (Goncalves, Gleize, Bott, Nowicki, Amiot, Lairon, et al., 2011; Goncalves, et al., 2014; Goncalves, Roi, Nowicki, Niot, & Reboul, 2014; Mensi, et al., 2014) absorption, as

well as to determine fat-soluble vitamin absorption sites (Goncalves, Roi, Nowicki, Dhaussy, Huertas, Amiot, et al., 2015). Our data showed that phytofluene bioavailability was higher than that of β-carotene and lycopene, phytoene bioavailability being intermediate. This is consistent with our previous observation that both phytoene and phytofluene were able to transfer more efficiently to mixed micelles during the digestion process compared to β-carotene and lycopene (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Stinco, & Melendez-Martinez, 2018). Our results are also in accordance with previous human studies showing that i/ lycopene and β-carotene postprandial responses are similar (O'Neill & Thurnham, 1998) and ii/ phytoene bioavailability is higher than that of lycopene (Moran, et al., 2016). Given the structural similarity between the studied molecules (all of them being linear carotenes), the difference between phytofluene bioavailability and that of lycopene is noteworthy. As indicated in our previous *in vitro* studies (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018), phytoene and phytofluene have a greater number of sigma bonds, where rotation is possible (G. Britton, Liaaen-Jensen, & Pfander, 2008), allowing them to fold more freely and to adopt less rigid shapes compared to lycopene (Lima, Sousa, Freitas, Ribeiro, de Sousa, & da Silva, 2017; Melendez-Martinez, Paulino, Stinco, Mapelli-Brahm, & Wang, 2014). This greater torsional capacity could translate into a better insertion between lipid molecules composing mixed micelles, which in turn could explain their greater micellization efficiency (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). This may also favor the binding with the membrane transporters responsible for their intestinal uptake, which in turn could explain their greater intestinal uptake efficiency (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Finally, this may facilitate their insertion into plasma membrane if a passive

diffusion occurs. The difference in their bioavailability can also be due to the fact that both phytoene and phytofluene were delivered in *Z*-forms while lycopene and β-carotene were delivered in *E*-forms. Indeed, although results concerning β-carotene are conflicting, *Z*-lycopene seems to be more bioavailable than *E*-lycopene (Desmarchelier & Borel, 2017).

We then assessed carotene accumulation in mouse intestinal mucosa along the duodenal-ileal axis and results were as follows: β-carotene + retinyl esters^{a} ≥ phytofluene^{ab} ≥ phytoene^{bc} ≥ lycopene^{c}. β-carotene accumulation profile displayed a shape along the duodenal-ileal axis of the intestine very close to that of retinol (Goncalves, et al., 2015). Additionally, β-carotene was also mostly converted in the proximal intestine, which is consistent with the proximal localization of the β-carotene 15,15' oxygenase-1 (BCO1) (Raghuvanshi, Reed, Blaner, & Harrison, 2015), Cytosolic Retinol Binding Protein Type II (CRBPII), retinal reductase and Lecithin-Retinol Acyltransferase (LRAT) (Herr, Wardlaw, Kakkad, Albrecht, Quick, & Ong, 1993). It is noteworthy that β-carotene postprandial response was not proportional to the important intestinal accumulation of β-carotene and its conversion products. This may be due to the fact that the intestine can store β-carotene from a first meal to release it during subsequent postprandial phases in humans (Borel, Mekki, Boirie, Partier, Alexandre-Gouabau, Grolier, et al., 1998). It would thus be interesting to perform another study during a longer period of time and with a second meal providing no carotenoids to further compare carotene release in the blood stream. Lycopene, which was recovered in low quantities compared to the other carotenes, accumulated similarly in the different parts of the intestine. Phytofluene and phytoene tended to accumulate in the distal part of the intestine, which has previously been observed for vitamin E and K (Goncalves, et al., 2015). Although transporter expression does not necessarily rely on protein level or activity, β-carotene accumulation interestingly showed a profile fairly similar to the gene expression of its identified transporters, and in particular to *CD36* expression. Indeed, both *Scarb1* and *CD36* were

mainly expressed in the proximal intestine, in accordance with previous work (Goncalves, Roi, Nowicki, Niot, & Reboul, 2014; Reboul, Soayfane, Goncalves, Cantiello, Bott, Nauze, et al., 2012). However, such similarity was not observed with the other carotenes. A first hypothesis to explain this discrepancy is that other carotenoid transporters, mostly expressed in the distal intestine, remain to be identified. A second hypothesis is linked to the fact that the accumulation of a given carotenoid within the mucosa is the result of both uptake and secretion of the molecule in the body or back to the lumen. Indeed, we previously showed that a fraction of phytoene and phytofluence is effluxed from the enterocyte to the apical media by Caco-2 cells (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Thus, the accumulation of the colorless carotenoids in the ileum may be due to a delayed or less efficient apical and/or basolateral secretion in this part of the intestine. However, in this case, the reason why such distal accumulation is not observed for β-carotene and lycopene is not known.

Altogether, our results show that phytofluene bioavailability in mice is higher than that of beta-carotene and lycopene after a gavage with standardized emulsions. Further investigations are now needed to confirm and understand why colorless carotenoids specifically accumulated in the distal intestine.

Conflict of interest:

A.J.M.M. is a member of the advisory board of IBR-Israeli Biotechnology Research, Ltd. (Yavne, Israel). Other authors declare no conflicts of interest or financial interest.

Acknowledgments:

This study was funded by both the Carnot Star Institute (consumables) and the Eurocaroten COST action CA15136 (Paula Mapelli-Brahm's stay in Marseille). A.J.M.M. and P.M.B.

301 acknowledged funding from the Andalusian Council of Economy, Innovation, Science and
302 Employment (project ref. CAROTINCO-P12-AGR-1287).
303

References

Borel, P., Lietz, G., Goncalves, A., Szabo de Edelenyi, F., Lecompte, S., Curtis, P., Goumidi, L., Caslake, M. J., Miles, E. A., Packard, C., Calder, P. C., Mathers, J. C., Minihane, A. M., Tourniaire, F., Kesse-Guyot, E., Galan, P., Hercberg, S., Breidenassel, C., Gonzalez Gross, M., Moussa, M., Meirhaeghe, A., & Reboul, E. (2013). CD36 and SR-BI Are Involved in Cellular Uptake of Provitamin A Carotenoids by Caco-2 and HEK Cells, and Some of Their Genetic Variants Are Associated with Plasma Concentrations of These Micronutrients in Humans. *J Nutr, 143*(4), 448-456.

Borel, P., Mekki, N., Boirie, Y., Partier, A., Alexandre-Gouabau, M. C., 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*(2), B133-B140.

Britton, G., & Khachik, F. (2009). Carotenoids in Food. In G. Britton, H. Pfander & S. Liaaen-Jensen (Eds.), *Carotenoids: Volume 5: Nutrition and Health*, (pp. 45-66). Basel: Birkhäuser Basel.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (2008). *Natural Functions* (Vol. 4). Basel - Boston: The Birkhäuser Verlag.

Desmarchelier, C., & Borel, P. (2017). Overview of carotenoid bioavailability determinants: From dietary factors to host genetic variations. *Trends in Food Science & Technology, 69*, 270-280.

Engelmann, N. J., Clinton, S. K., & Erdman, J. W., Jr. (2011). Nutritional aspects of phytoene and phytofluene, carotenoid precursors to lycopene. *Adv Nutr, 2*(1), 51-61.

Gleize, B., Steib, M., Andre, M., & Reboul, E. (2012). Simple and fast HPLC method for simultaneous determination of retinol, tocopherols, coenzyme Q(10) and carotenoids in complex samples. *Food Chem, 134*(4), 2560-2564.

330 Goncalves, A., Gleize, B., Bott, R., Nowicki, M., Amiot, M. J., Lairon, D., Borel, P., &
331 Reboul, E. (2011). Phytosterols can impair vitamin D intestinal absorption in vitro and
332 in mice. *Mol Nutr Food Res, 55 Suppl 2*, S303-311.
333 Goncalves, A., Gleize, B., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., Amiot, M. J., &
334 Reboul, E. (2013). Fatty acids affect micellar properties and modulate vitamin D
335 uptake and basolateral efflux in Caco-2 cells. *J Nutr Biochem, 24*(10), 1751-1757.
336 Goncalves, A., Margier, M., Roi, S., Collet, X., Niot, I., Goupy, P., Caris-Veyrat, C., &
337 Reboul, E. (2014). Intestinal scavenger receptors are involved in vitamin K1
338 absorption. *J Biol Chem, 289*(44), 30743-30752.
339 Goncalves, A., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., Amiot, M. J., & Reboul, E.
340 (2015). Fat-soluble vitamin intestinal absorption: absorption sites in the intestine and
341 interactions for absorption. *Food Chem, 172*, 155-160.
342 Goncalves, A., Roi, S., Nowicki, M., Niot, I., & Reboul, E. (2014). Cluster-determinant 36
343 (CD36) impacts on vitamin E postprandial response. *Mol Nutr Food Res, 58*(12),
344 2297-2306.
345 Herr, F. M., Wardlaw, S. A., Kakkad, B., Albrecht, A., Quick, T. C., & Ong, D. E. (1993).
346 Intestinal vitamin A metabolism: coordinate distribution of enzymes and CRBP(II). *J*
347 *Lipid Res, 34*(9), 1545-1554.
348 Jeffery, J. L., Turner, N. D., & King, S. R. (2012). Carotenoid bioaccessibility from nine raw
349 carotenoid-storing fruits and vegetables using an in vitro model. *J Sci Food Agric,*
350 *92*(13), 2603-2610.
351 Lima, I. T., Sousa, L., Freitas, R. D., Ribeiro, L. A., de Sousa, R. T., & da Silva, D. A. (2017).
352 A DFT study of a set of natural dyes for organic electronics. *Journal of Molecular*
353 *Modeling, 23*(12).

354 Mapelli-Brahm, P., Corte-Real, J., Melendez-Martinez, A. J., & Bohn, T. (2017).
355 Bioaccessibility of phytoene and phytofluene is superior to other carotenoids from
356 selected fruit and vegetable juices. *Food Chem, 229*, 304-311.
357 Mapelli-Brahm, P., Desmarchelier, C., Margier, M., Reboul, E., Melendez Martinez, A. J., &
358 Borel, P. (2018). Phytoene and Phytofluene Isolated from a Tomato Extract are
359 Readily Incorporated in Mixed Micelles and Absorbed by Caco-2 Cells, as Compared
360 to Lycopene, and SR-BI is Involved in their Cellular Uptake. *Mol Nutr Food Res,*
361 *62*(22), e1800703.
362 Mapelli-Brahm, P., Stinco, C. M., & Melendez-Martinez, A. J. (2018). Comparative study of
363 the bioaccessibility of the colorless carotenoids phytoene and phytofluene in powders
364 and pulps of tomato: microstructural analysis and effect of addition of sunflower oil.
365 *Food Funct, 9*(9), 5016-5023.
366 Melendez-Martinez, A. J., Mapelli-Brahm, P., Benitez-Gonzalez, A., & Stinco, C. M. (2015).
367 A comprehensive review on the colorless carotenoids phytoene and phytofluene. *Arch*
368 *Biochem Biophys, 572*, 188-200.
369 Melendez-Martinez, A. J., Paulino, M., Stinco, C. M., Mapelli-Brahm, P., & Wang, X. D.
370 (2014). Study of the time-course of cis/trans (Z/E) isomerization of lycopene,
371 phytoene, and phytofluene from tomato. *J Agric Food Chem, 62*(51), 12399-12406.
372 Mensi, A., Borel, P., Goncalves, A., Nowicki, M., Gleize, B., Roi, S., Chobert, J. M., Haertle,
373 T., & Reboul, E. (2014). Beta-lactoglobulin as a vector for beta-carotene food
374 fortification. *J Agric Food Chem, 62*(25), 5916-5924.
375 Moran, N. E., Clinton, S. K., & Erdman, J. W., Jr. (2013). Differential bioavailability,
376 clearance, and tissue distribution of the acyclic tomato carotenoids lycopene and
377 phytoene in mongolian gerbils. *J Nutr, 143*(12), 1920-1926.
378 Moran, N. E., Novotny, J. A., Cichon, M. J., Riedl, K. M., Rogers, R. B., Grainger, E. M.,
379 Schwartz, S. J., Erdman, J. W., Jr., & Clinton, S. K. (2016). Absorption and

Distribution Kinetics of the 13C-Labeled Tomato Carotenoid Phytoene in Healthy Adults. *J Nutr, 146*(2), 368-376.

Moussa, M., Landrier, J. F., Reboul, E., Ghiringhelli, O., Comera, C., Collet, X., Fröhlich, K., Böhm, V., & Borel, P. (2008). Lycopene absorption in human intestinal cells and in mice involves scavenger receptor class B type I but not Niemann-Pick C1-like 1. *J Nutr, 138*(8), 1432-1436.

O'Neill, M. E., & Thurnham, D. I. (1998). Intestinal absorption of β-carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction. *Br J Nutr, 79*, 149-159.

Raghuvanshi, S., Reed, V., Blaner, W. S., & Harrison, E. H. (2015). Cellular localization of beta-carotene 15,15' oxygenase-1 (BCO1) and beta-carotene 9',10' oxygenase-2 (BCO2) in rat liver and intestine. *Arch Biochem Biophys, 572*, 19-27.

Reboul, E. (2019). Mechanisms of Carotenoid Intestinal Absorption: Where Do We Stand? *Nutrients, 11*(4).

Reboul, E., & Borel, P. (2011). Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. *Prog Lipid Res, 50*(4), 388-402.

Reboul, E., Goncalves, A., Comera, C., Bott, R., Nowicki, M., Landrier, J. F., Jourdheuil-Rahmani, D., Dufour, C., Collet, X., & Borel, P. (2011). Vitamin D intestinal absorption is not a simple passive diffusion: evidences for involvement of cholesterol transporters. *Mol Nutr Food Res, 55*(5), 691-702.

Reboul, E., Soayfane, Z., Goncalves, A., Cantiello, M., Bott, R., Nauze, M., Terce, F., Collet, X., & Comera, C. (2012). Respective contributions of intestinal Niemann-Pick C1-like 1 and scavenger receptor class B type I to cholesterol and tocopherol uptake: in vivo v. in vitro studies. *Br J Nutr, 107*(9), 1296-1304.

405 Reboul, E., Trompier, D., Moussa, M., Klein, A., Landrier, J. F., Chimini, G., & Borel, P.
406 (2009). ATP-binding cassette transporter A1 is significantly involved in the intestinal
407 absorption of alpha- and gamma-tocopherol but not in that of retinyl palmitate in mice.
408 *Am J Clin Nutr, 89*(1), 177-184.
409 Ribaya-Mercado, J. D., Holmgren, S. C., Fox, J. G., & Russell, R. M. (1989). Dietary beta-
410 carotene absorption and metabolism in ferrets and rats. *J Nutr, 119*(4), 665-668.
411 Richelle, M., Sanchez, B., Tavazzi, I., Lambelet, P., Bortlik, K., & Williamson, G. (2010).
412 Lycopene isomerisation takes place within enterocytes during absorption in human
413 subjects. *Br J Nutr, 103*(12), 1800-1807.

414

415

416

417

418 **Figure legends**

419

420 **Figure 1: Postprandial plasma carotene concentrations in mice after gavage with**
421 **carotene-rich emulsions**

422 **A**: Phytoene (n=6). **B**: Phytofluene (n=5). **C**: Lycopene (n=6). **D**: β-Carotene and retinyl
423 esters (n=4).

424 Data are means ± SEM.

425

426 **Figure 2: Carotene contents of mouse intestinal segments after gavage with carotene-**
427 **rich emulsions**

428 **A**: Phytoene (n=6). **B**: Phytofluene (n=5). **C**: Lycopene (n=6). **D**: β-Carotene, newly-formed
429 retinol and retinyl esters (n=4).

430 Mouse intestines were harvested and cut in 5 segments of equal length, 6 h after gavage with
431 carotene-rich-emulsions. Data are means ± SEM.

432

433 **Figure 3: *CD36* and *Scarb1* expression along mouse intestine**

434 **A**: *CD36*. **B**: *Scarb1*.

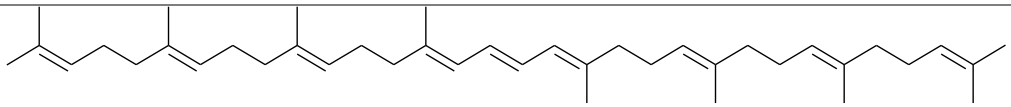
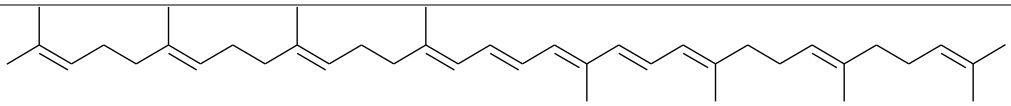
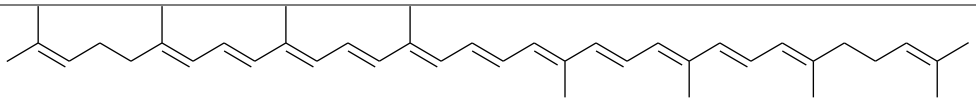
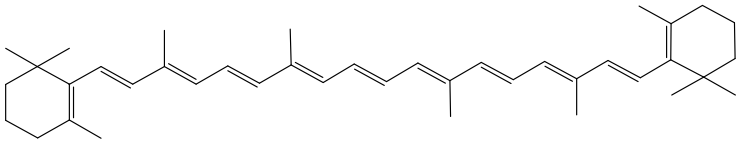
435 Relative gene expression profiles were analyzed by real-time PCR in mouse intestinal mucosa
436 (n=3). Values were normalized to 18S expression. Data are means ± SEM.

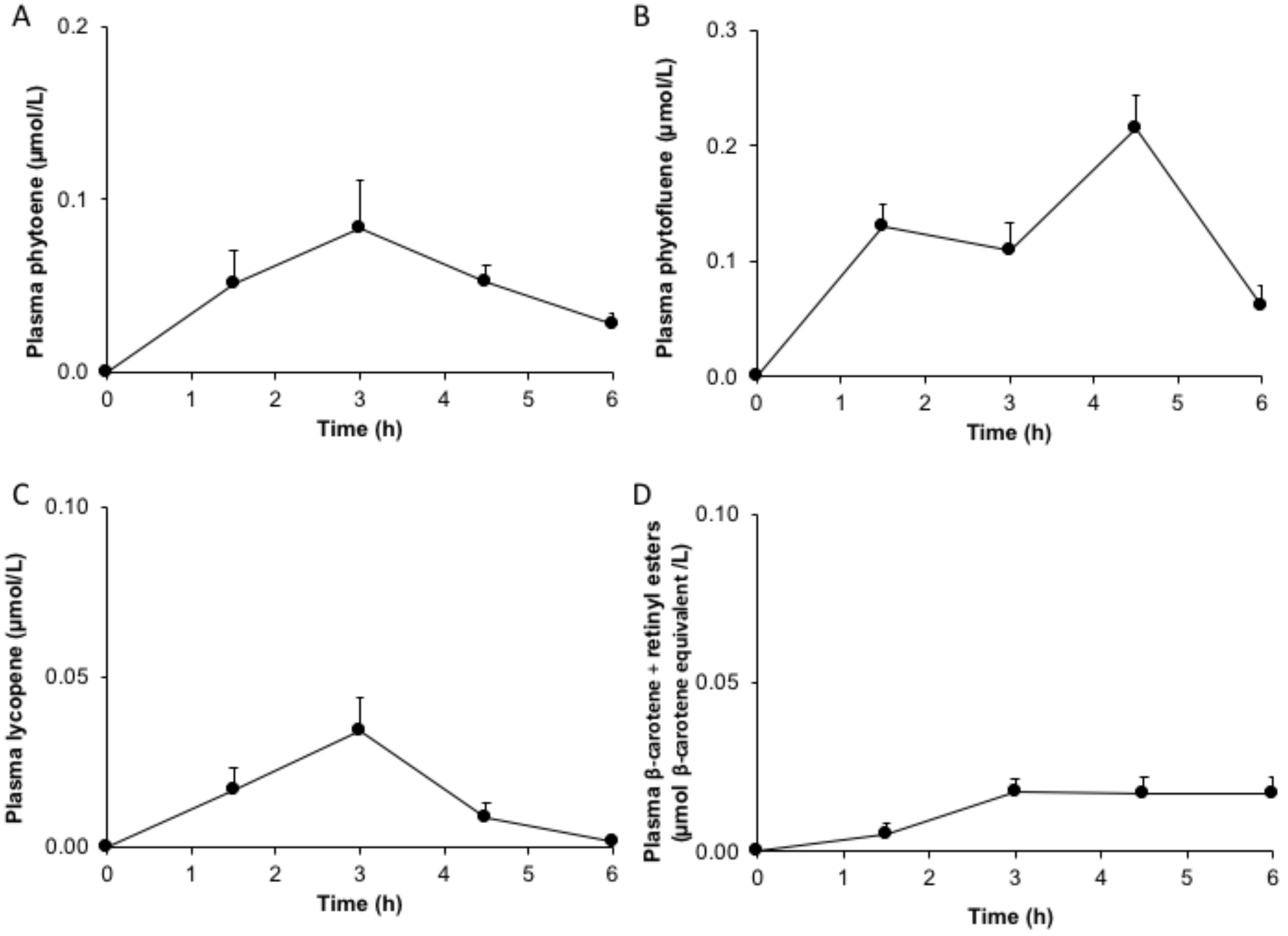
437

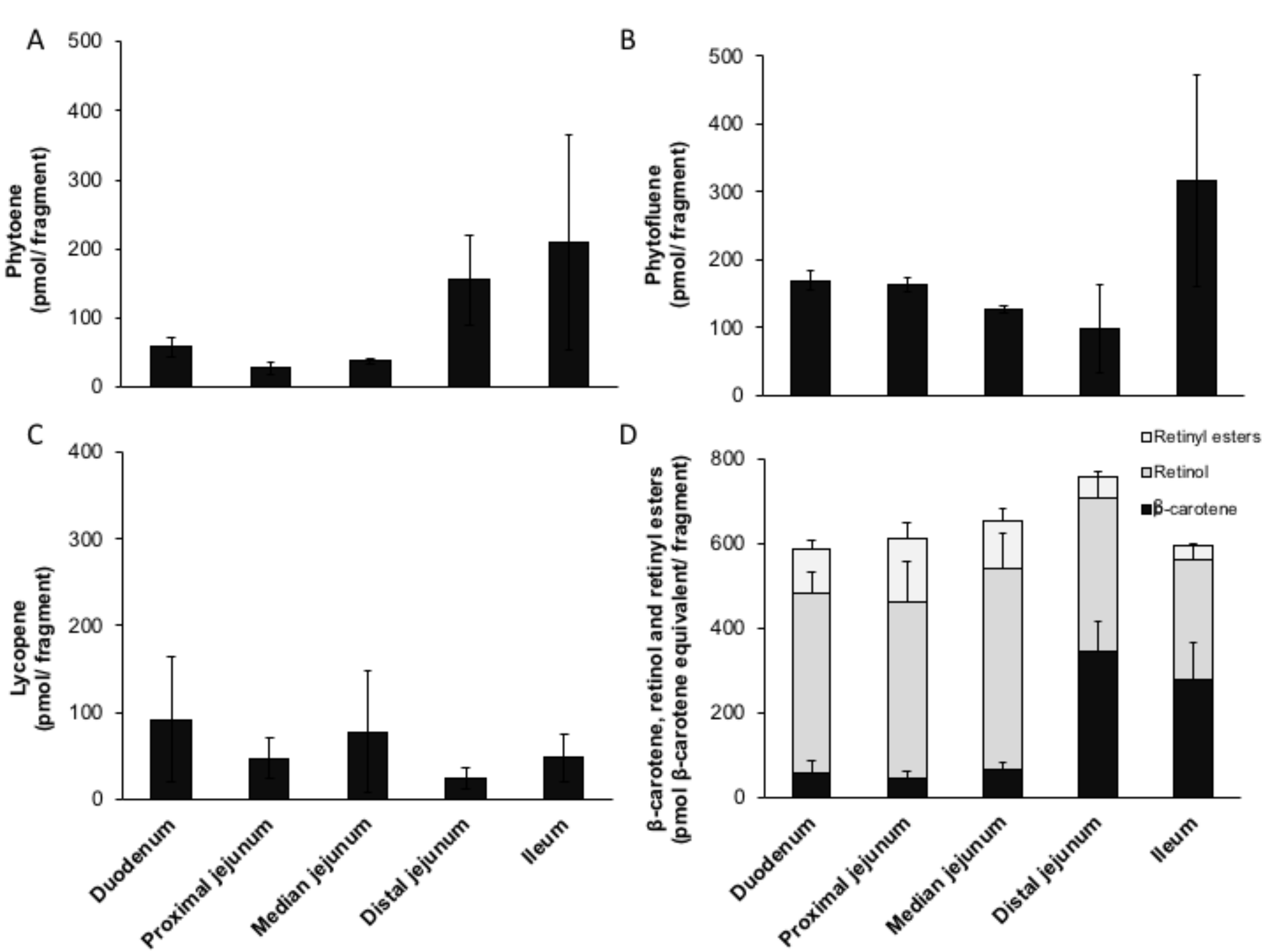
438

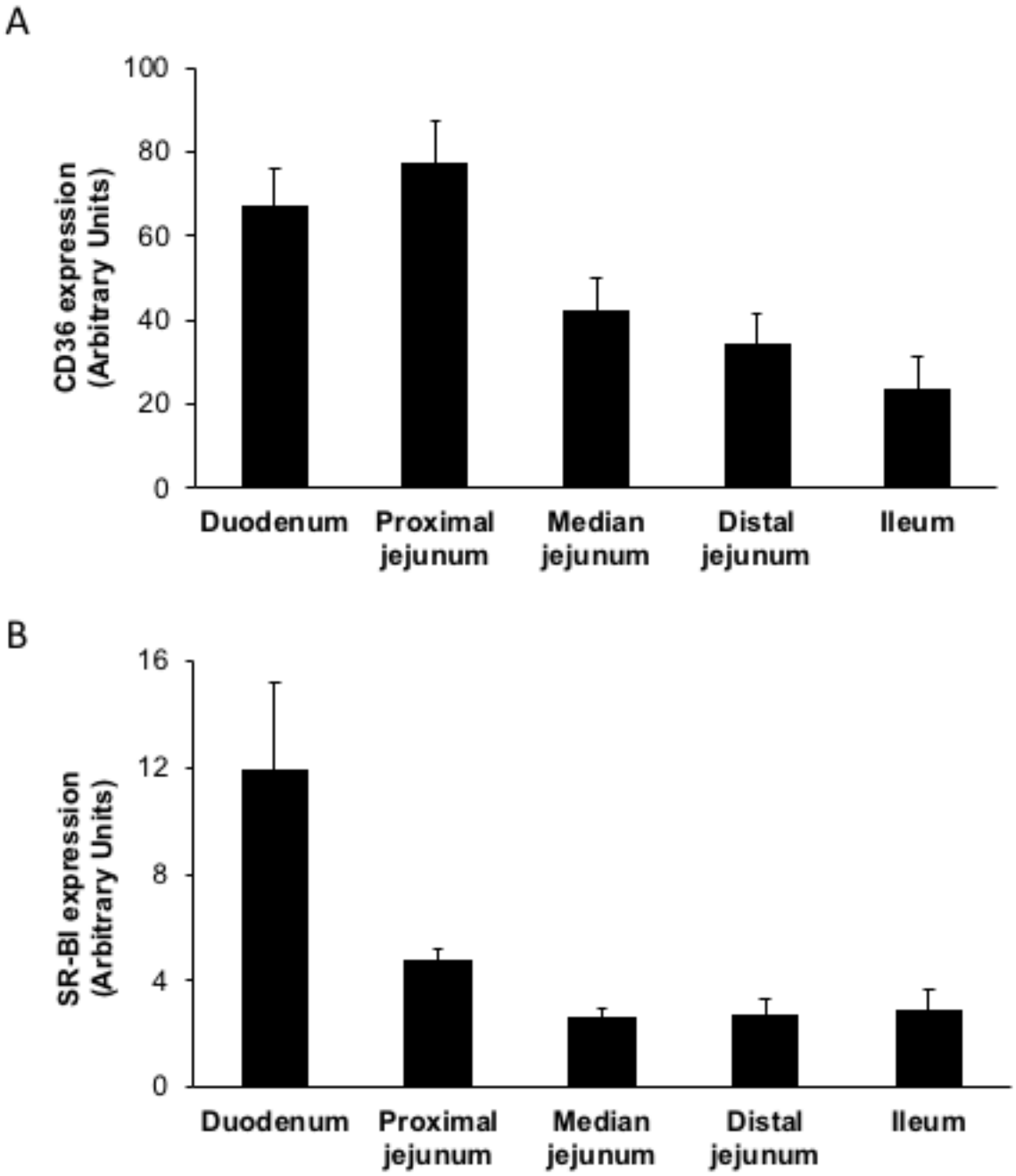
Tables

Table 1: Chemical structures of tomato carotenes

Carotene	Structure of the molecules used
Phytoene	
Phytofluene	
Lycopene	
β-Carotene	


A
Plasma phytoene (µmol/L)
0.2
0.1
0.0
0
1
2
3
4
5
6
Time (h)
B
Plasma phytofluene (µmol/L)
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Time (h)
C
Plasma lycopene (µmol/L)
0.10
0.05
0.00
0
1
2
3
4
5
6
Time (h)
D
Plasma β-carotene + retinyl esters (µmol β-carotene equivalent /L)
0.10
0.05
0.00
0
1
2
3
4
5
6
Time (h)


A
Phytoene (pmol/ fragment)
500
400
300
200
100
0
B
Phytofluene (pmol/ fragment)
500
400
300
200
100
0
C
Lycopene (pmol/ fragment)
400
300
200
100
0
D
β-carotene, retinol and retinyl esters (pmol β-carotene equivalent/ fragment)
800
600
400
200
0
Retinyl esters
Retinol
β-carotene
Duodenum
Proximal jejunum
Median jejunum
Distal jejunum
Ileum


A
100
80
60
40
20
0
CD36 expression (Arbitrary Units)
Duodenum
Proximal jejunum
Median jejunum
Distal jejunum
Ileum
B
16
12
8
4
0
SR-BI expression (Arbitrary Units)
Duodenum
Proximal jejunum
Median jejunum
Distal jejunum
Ileum