

# Comparison of the bioavailability and intestinal absorption sites of phytoene, phytofluene, lycopene and $\beta$ -carotene

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# 1 Comparison of the bioavailability and intestinal absorption sites of

# 2 phytoene, phytofluene, lycopene and β-carotene

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- 14
- Abbreviated running Title: Bioavailability and intestinal absorption sites of tomato
   carotenoids
- 17

#### 18 Abstract

20 The mechanisms of main tomato carotenes (phytoene, phytofluene, lycopene and  $\beta$ -carotene) intestinal absorption are still only partly understood. We thus compared carotene 21 22 bioavailability in mice after gavage with carotene-rich oil-in-water emulsions. We also 23 determined each carotene absorption profile along the duodenal-ileal axis of the intestine to 24 identify their respective absorption sites and compared these profiles with the gene expression 25 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  $\beta$ -carotene (areas 26 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 27 28 phytofluene, phytoene, lycopene and  $\beta$ -carotene, respectively).  $\beta$ -carotene was mostly 29 converted in the proximal and median intestine. Phytoene and phytofluene accumulation 30 tended to be more important in the distal intestine, which did not correlate with the proximal 31 expression of both *Scarb1* and *CD36*. Overall, these results highlight the high bioavailability 32 of phytofluene.

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

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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).

<sup>19</sup> 

#### 40 **1. Introduction**

41

42 Carotenoids represent a broad family of about 800 molecules synthetized by plants and 43 microorganisms, around 40 of which are found in significant amounts in human diets. Non-44 oxygenated and oxygenated carotenoids belong to the carotene and xanthophyll family, respectively. The main dietary carotenes are  $\beta$ - and  $\alpha$ -carotene, and lycopene. Interestingly, a 45 46 recent emphasis has been made on two other carotenes: phytoene and phytofluene (see for 47 review: (Reboul, 2019)). These molecules can be found in high (0.5-2 mg/100 g of fresh 48 weight) or very high (> 2 mg/100 g of fresh weight) concentrations (George Britton & 49 Khachik, 2009) in tomatoes, carrots, oranges and apricots, and are detectable in significant 50 amounts in both human plasma and tissues, where they might display specific health benefits 51 (Melendez-Martinez, Mapelli-Brahm, Benitez-Gonzalez, & Stinco, 2015). Indeed, phytoene 52 and phytofluene present particular properties due to their molecular structures. While other 53 dietary carotenoids contain at least 10 conjugated double bounds, phytoene and phytofluene 54 only present 3 and 5 conjugated double bounds, respectively (Table 1). These reduced 55 numbers of double bounds are responsible for their lack of color, their twisted shape, and 56 likely affect both their bioavailability and functional properties (Melendez-Martinez, Mapelli-57 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

65	(Moran, Clinton, & Erdman, 2013) and in humans (Moran, Novotny, Cichon, Riedl, Rogers,
66	Grainger, et al., 2016). However, no comprehensive comparison of the bioavailability of the 4
67	tomato carotenes, i.e. phytoene, phytofluene, lycopene and $\beta$ -carotene, has been conducted so
68	far <i>in vivo</i> .
69	To gain insight into carotene intestinal absorption, we thus i) compared the postprandial
70	responses of phytoene, phytofluene, lycopene and $\beta$ -carotene after gavage in mice and ii)

71 investigated their absorption sites along mouse intestine.

#### 3 **2.** Materials and methods

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#### 75 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, MelendezMartinez, & Bohn, 2017).

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#### 83 **2.2 Preparation of carotene-enriched emulsions for mouse experiments**

84 To deliver carotenes to mice, carotene-rich emulsions were prepared as previously described 85 (Reboul, Goncalves, Comera, Bott, Nowicki, Landrier, et al., 2011) with minor modifications. 86 An appropriate volume of the stock solution of each carotenoid was transferred to Eppendorf 87 tubes to obtain a final amount of 2 mg in each tube. Stock solution solvent was evaporated 88 under nitrogen. To facilitate the subsequent solubilisation of carotenoids in triolein, 15 µL of 89 hexane were added. The sample was vortexed 20 sec and then 1 mL of triolein were added. 90 The mixture was vortexed 1 min and the hexane was evaporated under nitrogen. After 1 min 91 of vortex, 1 mL of an aqueous solution (NaCl 0.9%, BSA 2% and phospholipids 0.1%) were 92 added. The sample was vortexed for 6 min and then probe-sonicated (20 sec two times) to 93 obtain homogenous and stable emulsions that were used within 10 min. Mice were force-fed 94 with 300  $\mu$ L of emulsions containing 250  $\mu$ g of either lycopene, phytoene, phytofluene or  $\beta$ -95 carotene.

#### 97 **2.3** Animals and sample collection

98 Six-week-old wild-type male C57BL/6 Rj mice (21-23 g) were purchased from Janvier 99 (Janvier, Le-Genest-St-Isle, France). The mice were housed in a temperature-, humidity- and 100 light-controlled room. They were given a standard chow diet and water *ad libitum*. Mice were 101 fasted overnight before each experiment. The protocol was approved by the ethics committee 102 of Marseille (agreement APAFIS#13473-20180209184003330 v3).

103 One week before the experiment, a blood sample ( $\approx 50 \ \mu$ L) was collected from the 104 submandibular vein at fast to evaluate mouse carotenoid status. On the day of the experiment, 105 the mice (n= 4 to 6) were force-fed with one of the carotene-rich emulsions and additional 106 blood samples ( $\approx 50 \ \mu$ L) were taken 1.5 h, 3h and 4.5 h after gavage. After 6 h, a last blood 107 sample was taken by intracardiac puncture under sevoflurane anesthesia. The plasma was 108 separated from the blood by centrifugation by using 3.8% sodium citrate as an anticoagulant. 109 The intestine of each animal was then quickly harvested after euthanasia by cervical 110 dislocation, and carefully rinsed with ice-cold PBS. The small intestine was cut into 5 equal 111 segments of 6 cm (representing the duodenum, the proximal – medial – distal jejunum and the 112 ileum) along a total length of 30 cm. All samples were suspended in 500 µL PBS, 113 homogenized and rapidly stored at -80 °C until analysis.

114

#### 115 **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

121 lycopene can be isomerized in the intestine (Richelle, Sanchez, Tavazzi, Lambelet, Bortlik, & 122 Williamson, 2010), both Z- and all-E-lycopene were quantified and results were pooled. Similarly, as  $\beta$ -carotene is highly converted into retinyl esters in mice (Ribaya-Mercado, 123 Holmgren, Fox, & Russell, 1989), retinyl oleate, linoleate, palmitate and stearate 124 125 concentrations were measured together with  $\beta$ -carotene in samples obtained after gavage with 126 the  $\beta$ -carotene-rich emulsion. Note that no retinyl esters were detected in the plasma of mice 127 that received nonprovitamin A carotene. Retinol deriving from  $\beta$ -carotene, i.e. [retinol 128 measured in mucosa samples after  $\beta$ -carotene gavage corrected for retinol present in mouse 129 mucosa after nonprovitamin A carotenoid gavage], was also evaluated in mouse intestine. 130 Retinol and retinyl esters recovered in these samples were then expressed in pmol equivalent 131 to  $\beta$ -carotene (1 mol of  $\beta$ -carotene =  $\frac{1}{2}$  mol retinol or  $\frac{1}{2}$  mol retinyl esters). After lipid 132 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. 133 134 The HPLC systems and methods were set up according to previous studies (Gleize, Steib, 135 Andre, & Reboul, 2012; Reboul, Trompier, Moussa, Klein, Landrier, Chimini, et al., 2009). 136 Each carotene was identified by retention time compared with pure standards.

137

#### 138 **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  $\mu$ Lite (Isogen Life Science, De Meern, The Netherlands). cDNAs were synthesized from 1  $\mu$ g 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 146as endogenous control in the comparative cycle threshold (CT) method. Primer sequences147were 18s [F: CGCCGCTAGAGGTGAAATTCT and R: CATTCTTGGCAAATGCTTTCG],148Scarb1 [F: AGCGCCAAGGTCATCATC and R: CTGCCTAACATCTTGGTCCTG], cd36149[F:CTTGTGTTTTGAACATTTCTGCTT150TTGTACCTATACTGTGGCTAAAATGAGA].

151

#### **2.6 Calculations and statistical analyses**

153 Carotenoid bioavailability was assessed by measuring the AUC of their postprandial plasma154 concentrations over 6 h, using the trapezoidal rule.

155 Results are expressed as means ± SEM. For ANOVA, normality of the residuals was checked 156 by Kolmogorov-Smirnov, using the Lilliefors correction, and Shapiro-Wilk tests while 157 equality of variances was checked by Levene's test. Differences in postprandial carotenoid 158 peak concentrations (C<sub>max</sub>) and intestinal carotenoid concentrations were analyzed using 159 ANOVA. Tukey's test, which maintains the family-wise error rate at alpha = 0.05, was used 160 as a post hoc for pairwise comparisons. Due to heteroscedasticity not resolved by data transformation, differences in carotenoid bioavailability were analyzed using the Kruskal-161 162 Wallis test. Dunn's test, with the Bonferroni adjustment was used as a post hoc test for 163 pairwise comparisons. Values of p<0.05 were considered significant. Statistical analyses were 164 performed using SPSS (version 20, SPSS Inc., Chicago, IL, USA).

#### 166 **3. Results**

167

#### 168 **3.1 Postprandial plasma carotene responses in mice**

169 We first assessed the appearance of carotenes in mouse blood compartment after gavage with 170 the different emulsions (Figure 1).  $\beta$ -carotene was largely recovered as retinyl esters (> 95%). 171 Phytoene, lycopene and  $\beta$ -carotene peaked in plasma around 3 h after gavage, while 172 phytofluene peaked around 4.5 h.

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

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#### **3.2** Carotene accumulation by mouse intestine

The accumulation of the different carotenes did not significantly differ from one segment to another (Figure 2).  $\beta$ -Carotene (i.e.  $\beta$ -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  $\beta$ -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  $\approx$  4-fold higher than that found in the duodenum.

190 Overall, the total quantity of the different carotenes in mouse intestinal mucosa were:  $3.13 \pm$ 

191 0.46<sup>a</sup> nmol for  $\beta$ -carotene (recovered in the form of  $\beta$ -carotene, retinol and retinyl esters),

192  $0.87 \pm 0.16^{ab}$  nmol for phytofluene,  $0.48 \pm 0.13^{bc}$  for phytoene and  $0.29 \pm 0.16^{c}$  for lycopene

- 193 (p=0.001).
- 194

#### 195 **3.3 Carotene transporter gene expression in mouse intestine**

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

201

202 The molecular mechanisms involved in the absorption of carotenoids are only partly 203 understood. Initially, Hollander and colleagues suggested that  $\beta$ -carotene was absorbed by 204 passive diffusion. However, studies have reconsidered this assumption and have shown that 205 carotenoid absorption mechanisms are more complex than previously described (Reboul & 206 Borel, 2011). In particular, we used Caco-2 cells and transfected Griptite cells to show that 207 the enterocyte uptake of provitamin-A carotenoids, including  $\beta$ -carotene, involved both SR-208 BI and CD36. This was comforted by the fact that genetic variations in the genes encoding 209 these membrane proteins were associated with plasma concentrations of provitamin A 210 carotenoids at a population level (Borel, Lietz, Goncalves, Szabo de Edelenvi, Lecompte, 211 Curtis, et al., 2013). Recently, we also showed that SR-BI, but not CD36, was involved in 212 phytoene and phytofluene uptake in these two cell models (Mapelli-Brahm, Desmarchelier, 213 Margier, Reboul, Melendez Martinez, & Borel, 2018). NPC1-like intracellular cholesterol transporter 1, NPC1L1, which is another fat-soluble micronutrient transporter (Reboul & 214 215 Borel, 2011), was neither involved in the transport of lycopene (Moussa, Landrier, Reboul, 216 Ghiringhelli, Comera, Collet, et al., 2008) nor in that of colorless carotenoids (Mapelli-217 Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Overall, SR-BI 218 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 226 well as to determine fat-soluble vitamin absorption sites (Goncalves, Roi, Nowicki, Dhaussy, 227 Huertas, Amiot, et al., 2015). Our data showed that phytofluene bioavailability was higher than that of  $\beta$ -carotene and lycopene, phytoene bioavailability being intermediate. This is 228 229 consistent with our previous observation that both phytoene and phytofluene were able to 230 transfer more efficiently to mixed micelles during the digestion process compared to  $\beta$ -231 carotene and lycopene (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Stinco, & Melendez-Martinez, 2018). Our results are also in accordance with 232 233 previous human studies showing that i/ lycopene and  $\beta$ -carotene postprandial responses are 234 similar (O'Neill & Thurnham, 1998) and ii/ phytoene bioavailability is higher than that of 235 lycopene (Moran, et al., 2016). Given the structural similarity between the studied molecules 236 (all of them being linear carotenes), the difference between phytofluene bioavailability and 237 that of lycopene is noteworthy. As indicated in our previous in vitro studies (Mapelli-Brahm, 238 Corte-Real, Melendez-Martinez, & Bohn, 2017; Mapelli-Brahm, Desmarchelier, Margier, 239 Reboul, Melendez Martinez, & Borel, 2018), phytoene and phytofluene have a greater 240 number of sigma bonds, where rotation is possible (G. Britton, Liaaen-Jensen, & Pfander, 241 2008), allowing them to fold more freely and to adopt less rigid shapes compared to lycopene 242 (Lima, Sousa, Freitas, Ribeiro, de Sousa, & da Silva, 2017; Melendez-Martinez, Paulino, 243 Stinco, Mapelli-Brahm, & Wang, 2014). This greater torsional capacity could translate into a 244 better insertion between lipid molecules composing mixed micelles, which in turn could 245 explain their greater micellization efficiency (Mapelli-Brahm, Corte-Real, Melendez-246 Martinez, & Bohn, 2017; Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez 247 Martinez, & Borel, 2018). This may also favor the binding with the membrane transporters 248 responsible for their intestinal uptake, which in turn could explain their greater intestinal 249 uptake efficiency (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018). Finally, this may facilitate their insertion into plasma membrane if a passive 250

251 diffusion occurs. The difference in their bioavailability can also be due to the fact that both 252 phytoene and phytofluene were delivered in *Z*-forms while lycopene and  $\beta$ -carotene were 253 delivered in *E*-forms. Indeed, although results concerning  $\beta$ -carotene are conflicting, *Z*-254 lycopene seems to be more bioavailable than *E*-lycopene (Desmarchelier & Borel, 2017).

255 We then assessed carotene accumulation in mouse intestinal mucosa along the duodenal-ileal axis and results were as follows:  $\beta$ -carotene + retinyl esters<sup>a</sup>  $\geq$  phytofluene<sup>ab</sup>  $\geq$  phytoene<sup>bc</sup>  $\geq$ 256 257 lycopene<sup>c</sup>.  $\beta$ -carotene accumulation profile displayed a shape along the duodenal-ileal axis of 258 the intestine very close to that of retinol (Goncalves, et al., 2015). Additionally,  $\beta$ -carotene 259 was also mostly converted in the proximal intestine, which is consistent with the proximal 260 localization of the β-carotene 15,15' oxygenase-1 (BCO1) (Raghuvanshi, Reed, Blaner, & 261 Harrison, 2015), Cytosolic Retinol Binding Protein Type II (CRBPII), retinal reductase and 262 Lecithin-Retinol Acyltransferase (LRAT) (Herr, Wardlaw, Kakkad, Albrecht, Quick, & Ong, 263 1993). It is noteworthy that  $\beta$ -carotene postprandial response was not proportional to the 264 important intestinal accumulation of  $\beta$ -carotene and its conversion products. This may be due 265 to the fact that the intestine can store  $\beta$ -carotene from a first meal to release it during 266 subsequent postprandial phases in humans (Borel, Mekki, Boirie, Partier, Alexandre-267 Gouabau, Grolier, et al., 1998). It would thus be interesting to perform another study during a 268 longer period of time and with a second meal providing no carotenoids to further compare 269 carotene release in the blood stream. Lycopene, which was recovered in low quantities 270 compared to the other carotenes, accumulated similarly in the different parts of the intestine. 271 Phytofluene and phytoene tended to accumulate in the distal part of the intestine, which has 272 previously been observed for vitamin E and K (Goncalves, et al., 2015). Although transporter 273 expression does not necessarily rely on protein level or activity,  $\beta$ -carotene accumulation 274 interestingly showed a profile fairly similar to the gene expression of its identified 275 transporters, and in particular to CD36 expression. Indeed, both Scarb1 and CD36 were 276 mainly expressed in the proximal intestine, in accordance with previous work (Goncalves, 277 Roi, Nowicki, Niot, & Reboul, 2014; Reboul, Soayfane, Goncalves, Cantiello, Bott, Nauze, et 278 al., 2012). However, such similarity was not observed with the other carotenes. A first 279 hypothesis to explain this discrepancy is that other carotenoid transporters, mostly expressed 280 in the distal intestine, remain to be identified. A second hypothesis is linked to the fact that 281 the accumulation of a given carotenoid within the mucosa is the result of both uptake and 282 secretion of the molecule in the body or back to the lumen. Indeed, we previously showed that 283 a fraction of phytoene and phytofluence is effluxed from the enterocyte to the apical media by 284 Caco-2 cells (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 285 2018). Thus, the accumulation of the colorless carotenoids in the ileum may be due to a 286 delayed or less efficient apical and/or basolateral secretion in this part of the intestine. 287 However, in this case, the reason why such distal accumulation is not observed for  $\beta$ -carotene 288 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.

293

#### 294 **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.

297

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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	<b>A</b> : <i>CD36</i> . <b>B</b> : <i>Scarb1</i> .
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 $\pm$ SEM.
437	

439 Tables

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# **Table 1: Chemical structures of tomato carotenes**





