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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

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

17

18 **Abstract**

19

20 The mechanisms of main tomato carotenes (phytoene, phytofluene, lycopene and β -carotene)
21 intestinal absorption are still only partly understood. We thus compared carotene
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
26 presented a significantly higher bioavailability compared to lycopene and β -carotene (areas
27 under the curve of 0.76 ± 0.09 vs. 0.30 ± 0.05 , 0.09 ± 0.05 and 0.08 ± 0.01 $\mu\text{mol/L}\cdot\text{h}$ for
28 phytofluene, phytoene, lycopene and β -carotene, respectively). β -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.

33

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

35

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

39

40 1. Introduction

41
42 Carotenoids represent a broad family of about 800 molecules synthesized 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,
45 respectively. The main dietary carotenes are β - and α -carotene, and lycopene. Interestingly, a
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).

58 Both phytoene and phytofluene were shown to display a higher bioaccessibility, i.e a better
59 incorporation into mixed micelles, compared to lutein, β -carotene and lycopene (Jeffery,
60 Turner, & King, 2012; Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017;
61 Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez, & Borel, 2018).
62 Phytofluene was also shown to be more absorbed by human intestinal cells in culture
63 compared to phytoene (Mapelli-Brahm, Desmarchelier, Margier, Reboul, Melendez Martinez,
64 & 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 β -carotene, has been conducted so
68 far *in vivo*.

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

72

73 2. Materials and methods

74

75 2.1 Chemicals

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

82

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 μ L 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 μ L of emulsions containing 250 μ g of either lycopene, phytoene, phytofluene or β -**
95 **carotene.**

96

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 ($\approx 50 \mu\text{L}$) 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 ($\approx 50 \mu\text{L}$) 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 $-80 \text{ }^\circ\text{C}$ until analysis.

114

115 **2.4 Carotene extraction and HPLC analysis**

116 Carotenes were extracted from blood or intestine samples using the method previously
117 described (Goncalves, Gleize, Roi, Nowicki, Dhaussy, Huertas, et al., 2013; Goncalves,
118 Margier, Roi, Collet, Niot, Goupy, et al., 2014). The internal standard was echinenone.
119 Phytofluene and phytoene were not reported to be metabolized in the intestine (Engelmann,
120 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.
123 Similarly, as β -carotene is highly converted into retinyl esters in mice (Ribaya-Mercado,
124 Holmgren, Fox, & Russell, 1989), retinyl oleate, linoleate, palmitate and stearate
125 concentrations were measured together with β -carotene in samples obtained after gavage with
126 the β -carotene-rich emulsion. Note that no retinyl esters were detected in the plasma of mice
127 that received nonprovitamin A carotene. Retinol deriving from β -carotene, i.e. [retinol
128 measured in mucosa samples after β -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 β -carotene (1 mol of β -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 μ L of mobile phase (20%
133 methyl-*tert*-butyl ether – 80% methanol). A volume of 180 μ L was used for HPLC analysis.
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**

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

146 as endogenous control in the comparative cycle threshold (CT) method. Primer sequences
147 were *I8s* [F: CGCCGCTAGAGGTGAAATTCT and R: CATTCTTGGCAAATGCTTTCG],
148 *Scarb1* [F: AGCGCCAAGGTCATCATC and R: CTGCCTAACATCTTGGTCCTG], *cd36*
149 [F: CTTGTGTTTTGAACATTTCTGCTT and R:
150 TTGTACCTATACTGTGGCTAAATGAGA].

151

152 **2.6 Calculations and statistical analyses**

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

155 Results are expressed as means \pm 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_{max}) 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
161 transformation, differences in carotenoid bioavailability were analyzed using the Kruskal-
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).

165

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). β -carotene was largely recovered as retinyl esters (> 95%).
171 Phytoene, lycopene and β -carotene peaked in plasma around 3 h after gavage, while
172 phytofluene peaked around 4.5 h.

173 The 4 carotenoids elicited significantly ($p < 0.001$) different C_{\max} (phytofluene: 0.22 ± 0.03^a ;
174 phytoene: 0.10 ± 0.02^b ; lycopene: 0.03 ± 0.02^{bc} ; β -carotene: 0.02 ± 0.00^c). They also
175 displayed significantly ($p = 0.002$) different bioavailabilities, with phytofluene exhibiting
176 significantly higher bioavailability compared to lycopene and β -carotene (0.76 ± 0.09^a , $0.30 \pm$
177 0.06^{ab} , 0.09 ± 0.06^b and 0.08 ± 0.01^b $\mu\text{mol/L.h}$ for phytofluene, phytoene, lycopene and β -
178 carotene, respectively).

179

180 **3.2 Carotene accumulation by mouse intestine**

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

188 example, the amount of phytoene recovered in the ileum was \approx 4-fold higher than that found
189 in the duodenum.

190 Overall, the total quantity of the different carotenes in mouse intestinal mucosa were: $3.13 \pm$
191 0.46^a nmol for β -carotene (recovered in the form of β -carotene, retinol and retinyl esters),
192 0.87 ± 0.16^{ab} nmol for phytofluene, 0.48 ± 0.13^{bc} for phytoene and 0.29 ± 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).

199

200 **Discussion**

201

202 The molecular mechanisms involved in the absorption of carotenoids are only partly
203 understood. Initially, Hollander and colleagues suggested that β -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 β -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 Edelenyi, 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
214 transporter 1, NPC1L1, which is another fat-soluble micronutrient transporter (Reboul &
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.

219 Despite a common SR-BI-dependent absorption pathway, carotene absorption efficiency
220 seems highly variable depending on the molecule considered. We thus performed a
221 comprehensive study to compare the bioavailability of the main tomato carotenes using a
222 postprandial model in mice. This model has previously been used to investigate both
223 carotenoids (Mensi, Borel, Goncalves, Nowicki, Gleize, Roi, et al., 2014) and fat-soluble
224 vitamins (Goncalves, Gleize, Bott, Nowicki, Amiot, Lairon, et al., 2011; Goncalves, et al.,
225 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
228 than that of β -carotene and lycopene, phytoene bioavailability being intermediate. This is
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 β -
231 carotene and lycopene (Mapelli-Brahm, Corte-Real, Melendez-Martinez, & Bohn, 2017;
232 Mapelli-Brahm, Stinco, & Melendez-Martinez, 2018). Our results are also in accordance with
233 previous human studies showing that i/ lycopene and β -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, &
250 Borel, 2018). Finally, this may facilitate their insertion into plasma membrane if a passive

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 β -carotene were
253 delivered in *E*-forms. Indeed, although results concerning β -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
256 axis and results were as follows: β -carotene + retinyl esters^a \geq phytofluene^{ab} \geq phytoene^{bc} \geq
257 lycopene^c. β -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, β -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 (CRBP II), retinal reductase and
262 Lecithin-Retinol Acyltransferase (LRAT) (Herr, Wardlaw, Kakkad, Albrecht, Quick, & Ong,
263 1993). It is noteworthy that β -carotene postprandial response was not proportional to the
264 important intestinal accumulation of β -carotene and its conversion products. This may be due
265 to the fact that the intestine can store β -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, β -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 phytofluene 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 β -carotene
288 and lycopene is not known.

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

293

294 **Conflict of interest:**

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

297

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303

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305

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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 \pm 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 \pm 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 \pm SEM.

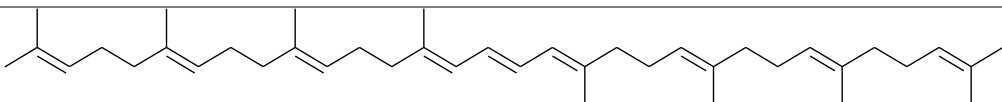
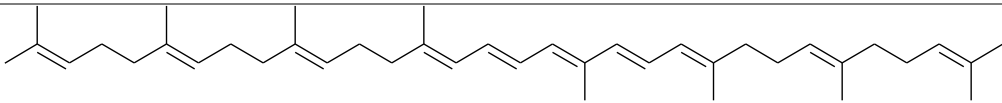
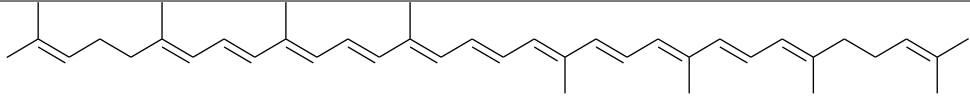
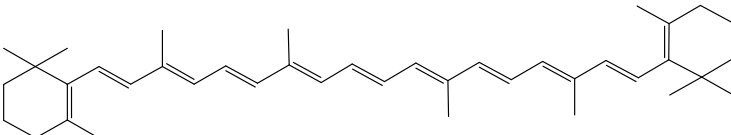
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439 **Tables**

440

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

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

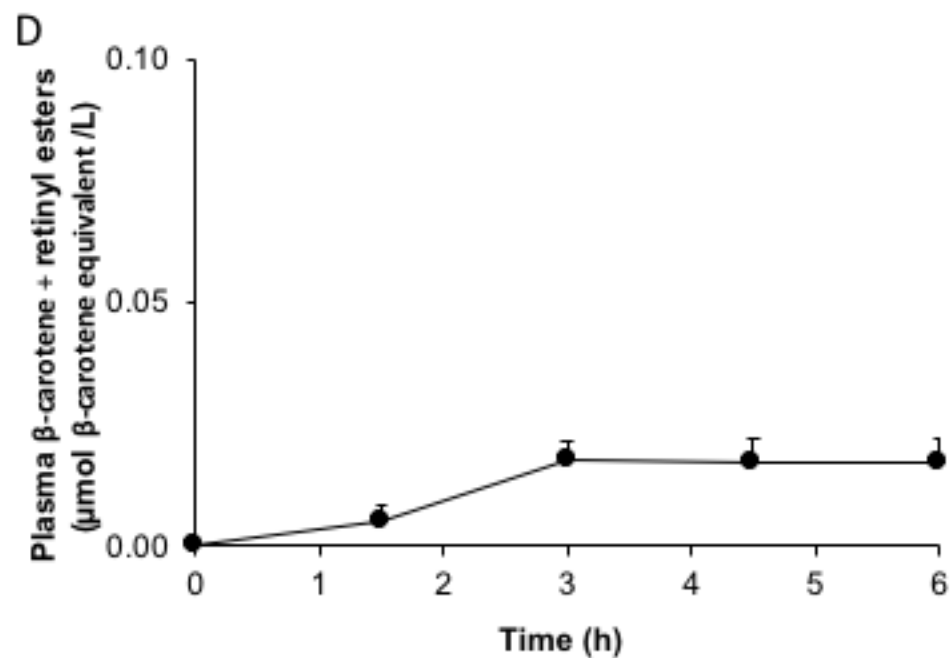
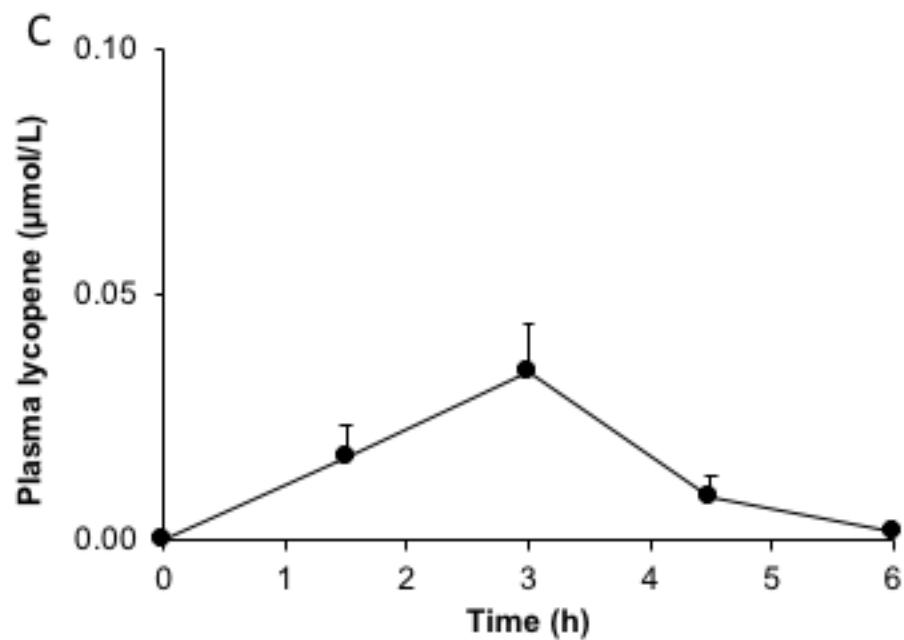
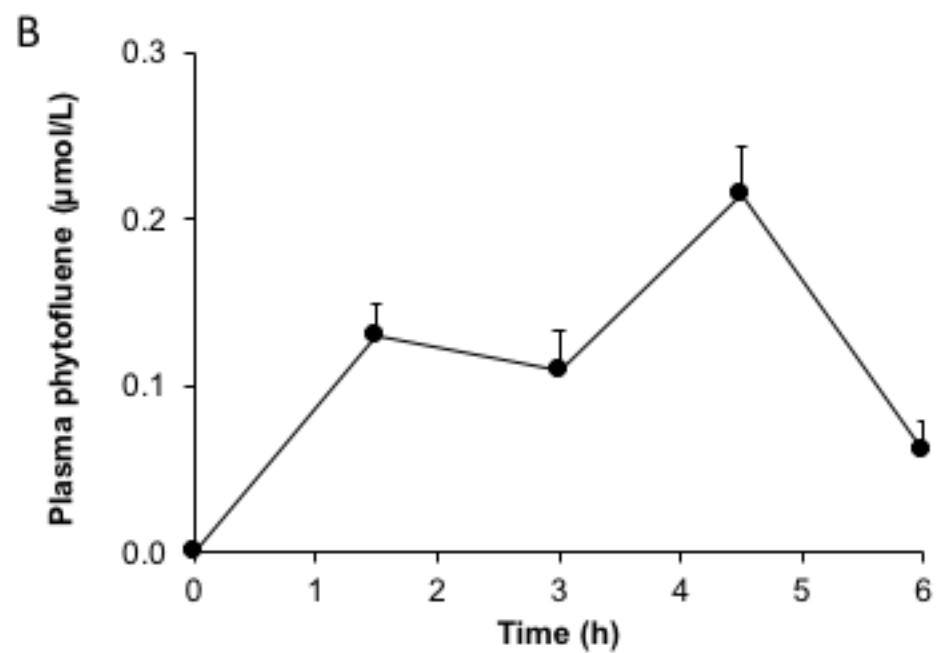
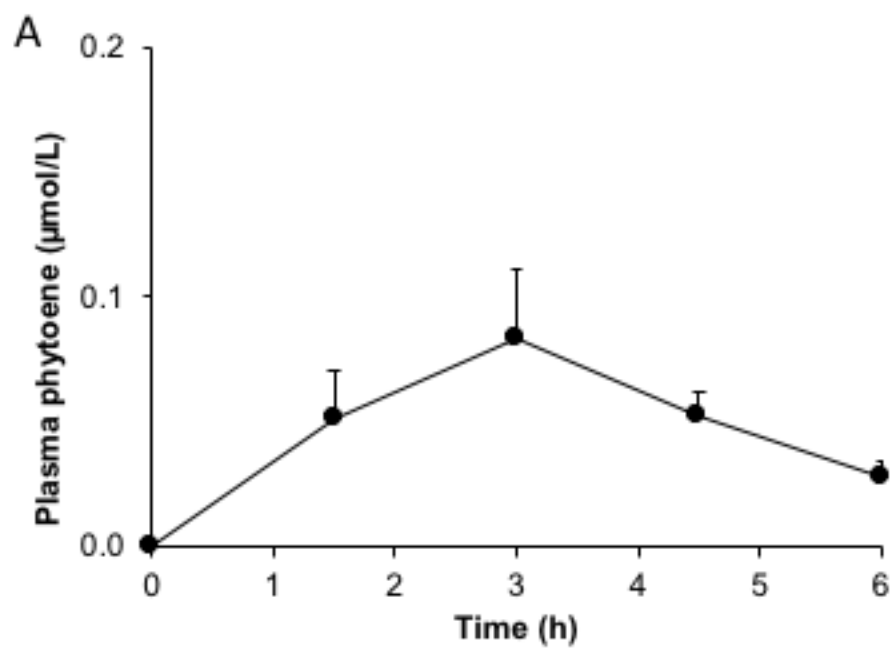
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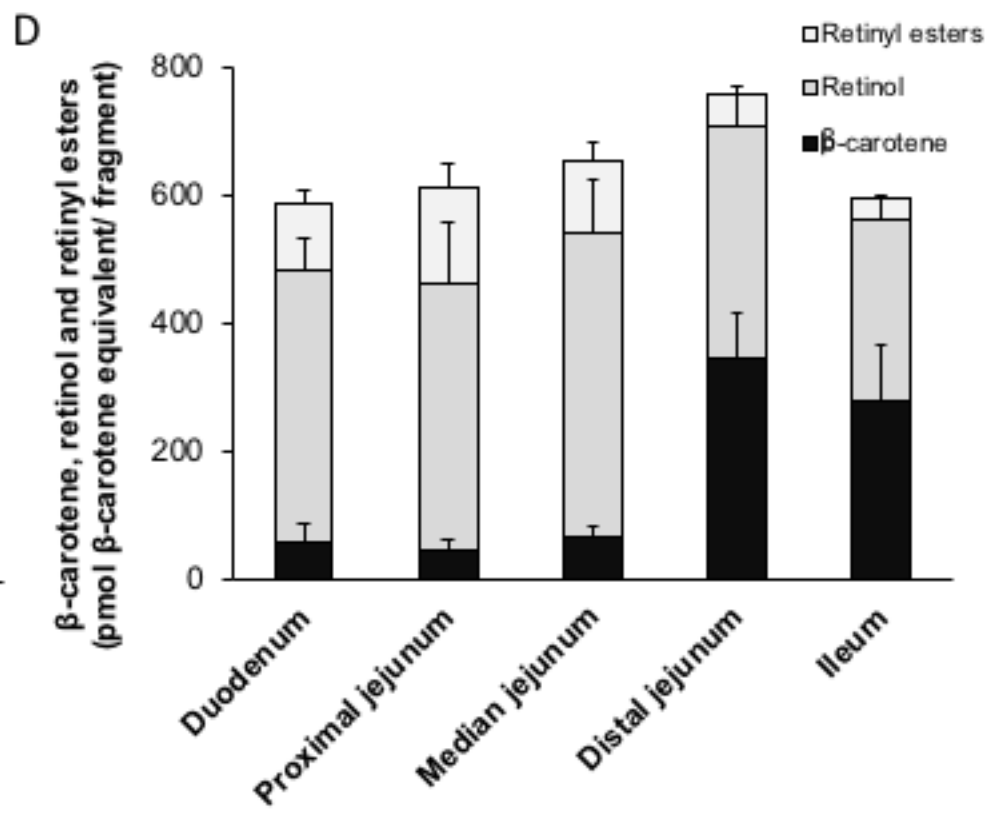
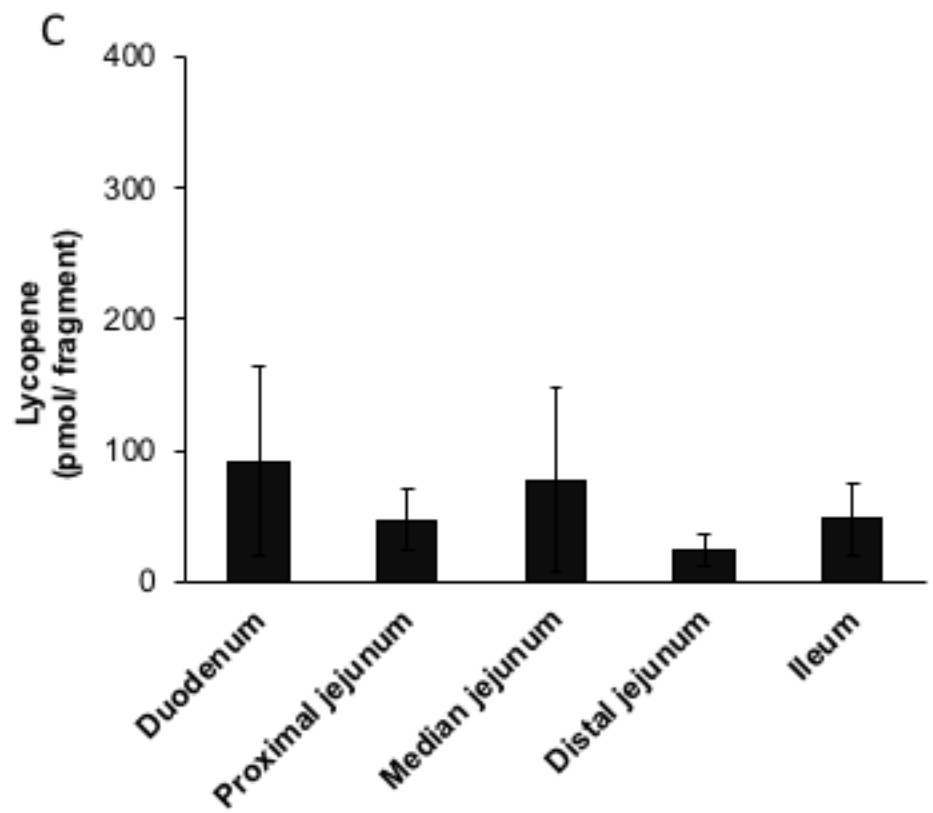
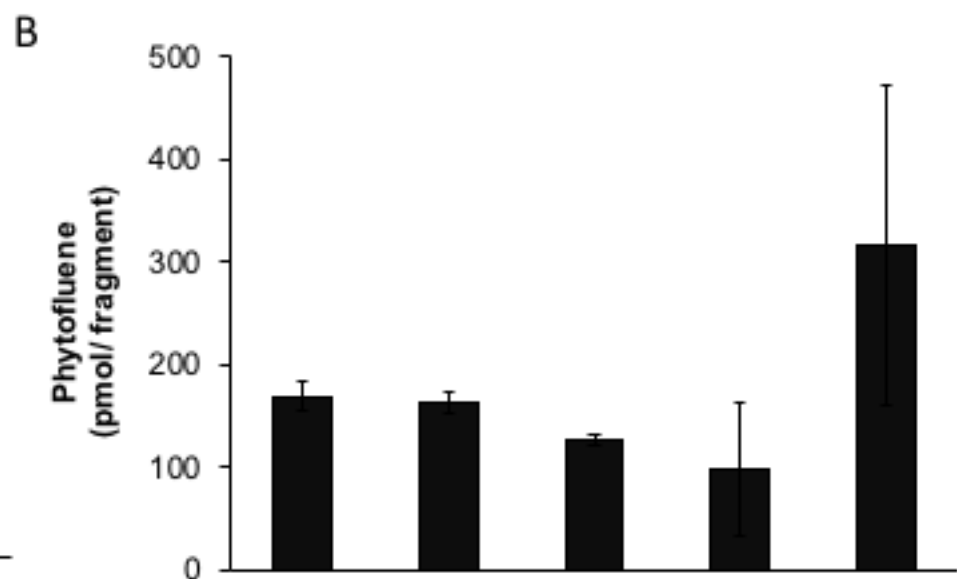
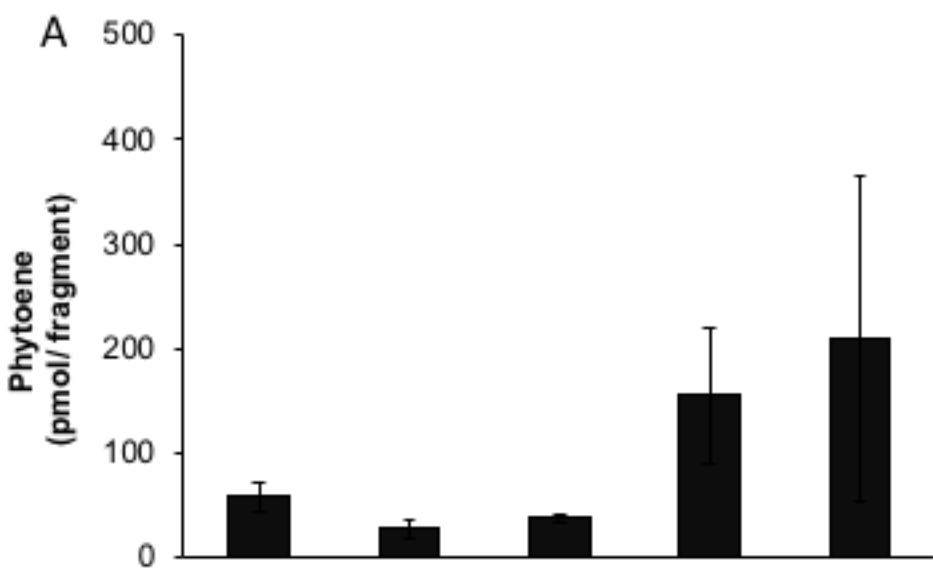
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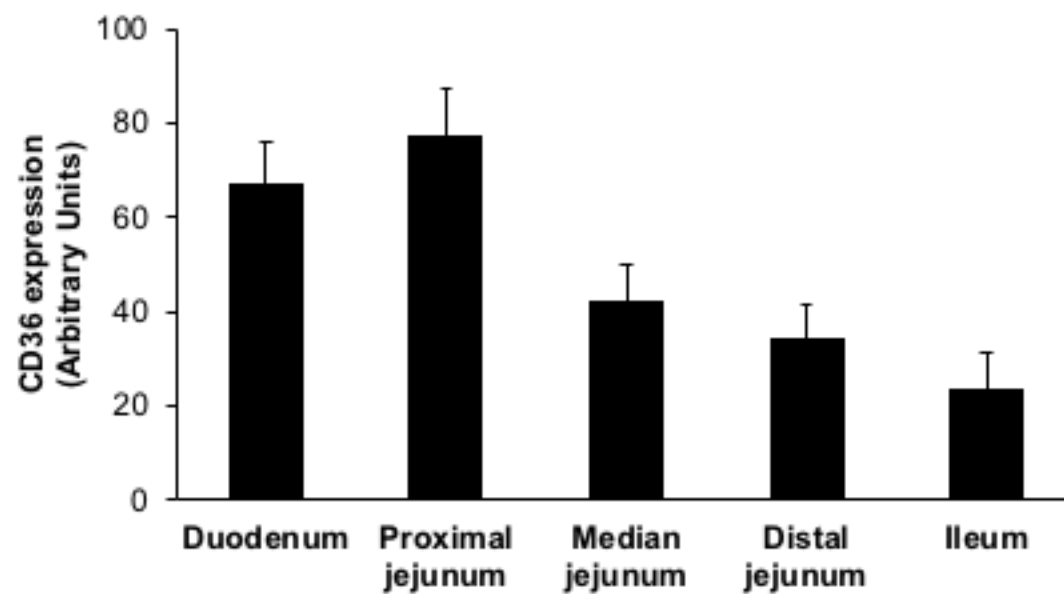
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