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1 Are Lutein, Lycopene, and  $\beta$ -carotene Lost through the Digestive Process?

2

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31 **Abstract**

32 The bioavailability of many carotenoids has been assessed, but little attention has been given to  
33 the metabolism of these antioxidant compounds during digestion. The isomerization and loss of  
34 lutein, lycopene, and  $\beta$ -carotene incorporated into a lipid-rich liquid meal was determined *in vitro*  
35 through the gastric, duodenal, and jejunal phases in the presence and absence of digestive enzymes,  
36 and in the presence and absence of known oxidizing agents often found in mixed meals  
37 (metmyoglobin in red meat and ferrous sulfate in supplemental iron). Carotenoids were quantitated  
38 using HPLC-PDA. In the absence of enzymes, lutein and lycopene were lost during earlier phases  
39 of the digestive process. In the presence of enzymes, lutein and lycopene were robust through the  
40 gastric and duodenal phases, with statistically significant losses of 40% and 20%, respectively,  
41 observed only during the jejunal phase. Regardless of the presence or absence of enzymes, an  
42 initial 25% of  $\beta$ -carotene was lost during the gastric phase, but no further loss was observed.  
43 Ferrous sulfate had no significant impact on any carotenoid level. Metmyoglobin had no impact  
44 on lutein, but significantly reduced lycopene and  $\beta$ -carotene levels by 30% and 80%, respectively,  
45 by the end of the jejunal phase. No significant isomerization was observed between the initial and  
46 jejunal phases for any of the carotenoids.

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## 48 1. Introduction

49 The consumption of carotenoid-containing foods has been associated with a reduced risk of  
50 developing a number of different types of diseases, such as various types of cancer<sup>1</sup> and  
51 cardiovascular disease.<sup>2</sup> The contribution of oxidative stress to these and other chronic conditions  
52 has been well established.<sup>3-5</sup> Thus, for many years, the prevailing hypothesis was that carotenoids  
53 absorbed from carotenoid-containing foods exerted antioxidant and singlet oxygen quenching  
54 effects *in vivo*, ultimately leading to a reduction in chronic diseases.<sup>6-8</sup>  
55 However, low carotenoid concentrations in areas where oxidative damage is a concern<sup>9</sup>, as well as  
56 inconsistent results from high-dose clinical studies, have called into question the plausibility of  
57 this hypothesis.<sup>10-12</sup>

58 In contrast, higher carotenoid concentrations found in the lumen of the gastrointestinal tract  
59 (i.e. after the consumption of a carotenoid-containing meal) more convincingly support the theory  
60 that these compounds may act as antioxidants in the gut.<sup>13,14</sup> In fact, work from our group<sup>15-18</sup> and  
61 others<sup>19,20</sup> has demonstrated the capacity of carotenoids to confer protection to unsaturated fatty  
62 acids under gastric and micellar (i.e. duodenal-like) conditions. Carotenoids also have a  
63 notoriously low bioaccessibility<sup>21</sup> and bioavailability<sup>22</sup> relative to other fat-soluble antioxidants  
64 like tocopherols<sup>23</sup> and omega-3-fatty acids.<sup>24</sup> It is possible that at least a portion of this low  
65 bioavailability may be due to loss of the carotenoid via oxidation during the digestive process.  
66 Indeed, carotenoids are known to be sensitive to increased temperature, pro-oxidative species, and  
67 an acidic pH, three factors found during digestion.<sup>13,14</sup> Furthermore, foods are consumed and co-  
68 consumed in a multitude of combinations. The essential metal iron is present in various oxidation  
69 states and complexes in the diet, and has also been shown to be a strong carotenoid oxidizing

70 agent.<sup>15</sup> The oxidation effect of iron may be perpetuated in the presence of unsaturated fatty acids,  
71 where lipid peroxidation products can ultimately co-oxidize carotenoids in solution.<sup>16</sup>

72 There is still no prevailing consensus on the mechanism(s) of action of non-provitamin A  
73 carotenoids once absorbed in the human body. More recent theories suggest that carotenoids may  
74 serve as “pro-bioactives”, i.e. the parent carotenoid consumed from a fruit or vegetable may be  
75 chemically or enzymatically converted into a biologically active metabolite(s) that ultimately  
76 exerts a disease-protective effect.<sup>25,26</sup> This hypothesis is further supported by the fact that non-  
77 provitamin A metabolites of the most commonly consumed carotenoids, i.e.  $\beta$ -carotene, lycopene,  
78 and lutein, have been identified in blood plasma and various fruits, vegetables, and food  
79 products.<sup>27–29</sup> It is not clear if these products were 1) absorbed directly from the foods themselves,  
80 2) formed during digestion, 3) formed after parent carotenoid absorption, or a combination thereof.

81 To better understand the fate of carotenoids during digestion, *in vitro* methods were employed  
82 to study carotenoid stability after each phase (initial, post-gastric, post-duodenal, and post-jejunal).  
83 Three of the most widely consumed carotenoids were chosen for study: lutein,  $\beta$ -carotene, and  
84 lycopene. Experiments were carried out both with and without digestive enzymes to better  
85 understand their influence. Finally, the impact of different dietary iron sources (i.e. ferrous sulfate  
86 commonly prescribed as an iron supplement, and metmyoglobin as the primary source of oxidized  
87 iron in red meat) were also investigated.

## 88 **2. Materials & Methods**

89 *2.1 Materials* – For digestion experiments, food-grade lutein powder (product number 1EAA6165;  
90 69% lutein by weight, as determined by molar extinction coefficient of all-*trans* lutein and HPLC-  
91 PDA analysis; geometrical isomer breakdown: 95% all-*trans*- and 5% *cis*-lutein) and food-grade  
92 lycopene-containing oleoresin (product number 1EAA9272; 4.5% lycopene by weight as

93 determined by molar extinction coefficient of all-*trans*-lycopene and LC-PDA analysis;  
94 geometrical isomer breakdown: 78% as all-*trans*-, 11% 5-*cis*- and 11% other-*cis*-lycopene) were  
95 kindly donated by Simona Birtic of Naturex (Montfavet, France). Fluka brand  $\beta$ -carotene ( $\geq 97\%$   
96 pure; geometrical isomer breakdown: 96% all-*trans*- and 4% *cis*- $\beta$ -carotene) from Sigma-Aldrich  
97 (Saint-Quentin-Fallavier, France) was used both for digestion experiments and for analytical  
98 quantitation. White granular sugar and 100% sunflower oil were purchased from a local  
99 supermarket (Auchan, Avignon, France). Food-grade phospholipid (Ovolife IF 50, containing 50%  
100 w/w phospholipid derived from egg yolk with a maltodextrin excipient) was kindly donated by  
101 Lecico, Inc. (Hamburg, Germany). Deionized water was obtained through filtration via a Millipore  
102 Q-Plus. HPLC grade methyl *tert*-butyl ether (MTBE) and LC-MS grade methanol were purchased  
103 from Fisher Scientific (Illkirch, France). Ammonium acetate ( $\geq 98\%$  pure, reagent grade), pepsin  
104 (pepsin A, from porcine gastric mucosa), bile extract (porcine origin), pancreatin (from porcine  
105 pancreas), mucin (type III, from porcine stomach),  $\alpha$ -amylase (from *Bacillus subtilis*), myoglobin  
106 ( $\geq 90\%$  from equine heart), sodium chloride (NaCl), sodium citrate tribasic dehydrate, sodium  
107 bicarbonate, calcium chloride dihydrate, potassium phosphate, disodium  
108 ethylenediaminetetraacetic acid (EDTA); Fluka brand potassium chloride, Extrasynthese brand  
109 lycopene (analytical grade), and Sigma brand pyrogallol ( $>99\%$ ) were purchased from Sigma-  
110 Aldrich (Saint-Quentin-Fallavier, France). Hydrochloric acid (HCl) was purchased from VWR.  
111 Tardyferon<sup>®</sup> was used as the source of ferrous sulfate (Boulogne, France). O-TRENSEX<sup>®</sup> (used  
112 to chelate metmyoglobin<sup>30</sup>) was a kind gift from Gisele Gellon.

113 *2.2 Carotenoid in oil preparation for the test meals* –  $\beta$ -Carotene (2 mg) or powdered lutein (3.1  
114 mg) were added to 0.6 g sunflower oil, while the lycopene oleoresin (63 mg) was combined with  
115 0.84 g sunflower oil. The oil mixtures were stirred overnight in the absence of light and under

116 argon. Note that the quantity of each carotenoid-containing product mixed with oil was dosed to  
117 deliver approximately 1.0 mg of carotenoid in 0.3 g oil.

118 *2.3 Test meal preparation* - Quantities of the test meal ingredients are shown in Table 1. Water  
119 and sugar were first mixed together in a beaker until the sugar was dissolved. The phospholipid  
120 containing powder (Ovolife IF 50) was added to the mixture and homogenized using a Heidolph  
121 Silent Crusher M (Schwabach, Germany) operated at 24,000 rpm for 2 min. Sunflower oil was  
122 then added and the mixture was again homogenized using the same conditions, followed by probe  
123 sonication using a Q700 QSonica (Newton, USA) with 40% amplitude and 30 sec sonication  
124 followed by 30 sec rest, repeated 8 x in sequence. After the removal of 18 mL of solution (which  
125 served to provide 3 “control” meals), the carotenoid in oil was added to the test meal remaining in  
126 the beaker. The meal was again homogenized and probe sonicated as described before.

127 *2.4 Ferrous sulfate ( $Fe^{II}$ ) solution* – A Tardyferon<sup>®</sup> tablet containing 80 mg of iron was powdered,  
128 and 20% of the final powder weight was added to 45 mL of a 0.9% aq. NaCl solution. The solution  
129 was homogenized for 30 sec and then bath sonicated for 10 min to enhance dissolution (final  
130 concentration = 5.7 mM  $Fe^{II}$ /6 mL).

131 *2.5 Metmyoglobin ( $MbFe^{III}$ ) solution* – The Sigma product myoglobin ( $MbFe^{II}$ ) was dissolved in  
132 water and tested via UV-Visible spectrometry to indeed confirm by  $\lambda_{max}$  that the product had been  
133 completely oxidized to metmyoglobin ( $MbFe^{III}$ )<sup>31</sup>.  $MbFe^{III}$  (21 mg) was dissolved in 10 mL of a  
134 0.9% aq. NaCl solution (final concentration = 40  $\mu$ M  $MbFe^{III}$  /2 mL).

135 *2.6 Inhibitor solutions* – Inhibitor solutions were prepared to halt any further chemical degradation  
136 of the digesta samples during extraction. The pyrogallol solution (used to quench samples of the  
137 digesta of carotenoid alone) was prepared by dissolving 125 mg pyrogallol in 25 mL water. The  
138  $Na_2EDTA$  solution (to quench the carotenoid +  $Fe^{II}$  digesta) was made by dissolving 140 mg

139 Na<sub>2</sub>EDTA and 100 mg pyrogallol in 20.2 mL of water. The O-TRENTOX® solution (to quench  
140 carotenoid + MbFe<sup>III</sup> digesta) was made by dissolving 8.6 mg O-TRENTOX® and 100 mg  
141 pyrogallol in 20.2 mL of water.

142 *2.7 Digestion with enzymes* – Following the initial *in vitro* digestion method of Garret et al.<sup>32</sup>,  
143 modifications were made as previously described<sup>33</sup>, as well as the addition of a jejunal phase. Each  
144 digestive condition with carotenoid was tested in triplicate, and the test beakers were placed on a  
145 magnetic stir plate in an oven (in the absence of light) at 37 °C during each phase. The digestions  
146 were prepared by adding 6 mL of test meal (delivering 80 µg carotenoid) to 32 mL of a 0.9% aq.  
147 NaCl solution (carotenoid alone), 30 mL of a 0.9% aq. NaCl solution containing 2 mL of the  
148 MbFe<sup>III</sup> solution (carotenoid + MbFe<sup>III</sup>), or 26 mL of a 0.9% aq. NaCl solution containing 6 mL of  
149 the Fe<sup>II</sup> solution (carotenoid + Fe<sup>II</sup>). The buccal phase (10 min), gastric and duodenal phases (each  
150 30 min) were mimicked as previously described.<sup>33</sup> The jejunal phase was mimicked by adjusting  
151 the pH to 7 by adding ~1.2 mL 0.9M aq. NaHCO<sub>3</sub> and digesting for 30 min. In addition, during the  
152 duodenal and jejunal phases, the beaker headspace was backfilled with argon to displace any air  
153 and thus any dioxygen molecules. Samples (0.5 mL) were taken immediately after the test meal  
154 was added to the digestion beaker (t = 0), and after the gastric, duodenal, and jejunal phases, and  
155 quenched with an equivalent volume of their respective inhibitor solutions.

156 *2.8 Digestion without enzymes*– The procedures were followed as described above, with the  
157 exception that the respective digestion buffers alone were added in place of the buffers with  
158 enzymes.

159 *2.9 Sample extraction* – To each sample already combined with inhibitor solution, methanol (0.5  
160 mL) was added and the vial was shaken for 1 minute. Next, hexane was added (2 mL), the sample  
161 vortexed for 1 min. The upper hexane phase was transferred to a clean glass vial, and



162 dichloromethane (2 mL) was then added to the remaining sample. The sample was again vortexed  
163 (1 min), and then centrifuged for 2 min at 3,000 rpm (Jouan MR1822, ThermoFisher Scientific,  
164 Walham, MA, U.S.A.) to induce a clean phase separation. The lower dichloromethane phase was  
165 removed and pooled with the hexane extract and dried under a stream of argon at 30°C. Dried  
166 extracts were stored at -20°C for no more than 2 days before analysis.

167 *2.8 HPLC Analysis* – Extracts were redissolved in 50 µL MTBE, to which 50 µL of MeOH was  
168 added and samples were briefly sonicated (< 5 sec) in a sonication bath for dissolution. Samples  
169 were analyzed using an HP1100 (Agilent Technologies, Santa Clara, CA, U.S.A.) HPLC system  
170 equipped with a photodiode array detector. Lutein and β-carotene samples were separated using a  
171 YMC C30 column (150 mm x 2.0 mm, 3 µm particle size) using a previously published liquid  
172 chromatography method<sup>34</sup> employing solvent A (80:18:2 MeOH/water/2% aq. ammonium acetate)  
173 and solvent B (20:78:2 MeOH/MTBE/2% aq. ammonium acetate), but halting the gradient at 26  
174 min and injecting 10 µL. Note that this method separated all-*trans*-β-carotene and all-*trans*-lutein  
175 from *cis* geometrical isomers (9-*cis*-β-carotene was putatively determined by coincident PDA  
176 spectra and elution order just after all-*trans*-β-carotene<sup>35</sup>, while all other *cis*-isomers of β-carotene  
177 and all *cis* isomers of lutein were determined by PDA spectra and elution prior to the all-*trans*  
178 configuration). Lycopene samples were separated using a method designed to partially separate 5-  
179 *cis*-lycopene (which elutes just after the all-*trans* configuration on a C30 column<sup>36</sup>) from all-*trans*-  
180 lycopene, as well as other *cis*-isomers (which elute prior to the all-*trans* configuration). A YMC  
181 C30 column (150 mm x 4.6 mm, 3 µm particle size) column was employed using the same solvent  
182 system as above, with the following gradient: beginning at 40% B, increasing linearly to 80% B  
183 over 23 min, holding at 80% B for 1 min, returning to 40% B over 3 min. The column was held at  
184 a cooler temperature of 20°C (which afforded better separation of the lycopene isomers<sup>37</sup>), the

185 flow rate was 1.3 mL/min, and 10  $\mu$ L samples were injected. Carotenoids were quantitated using  
186 external calibration curves generated from authentic standards by integrating the peak areas at 445  
187 nm (lutein), 450 nm ( $\beta$ -carotene), and 471 nm (lycopene). Total carotenoid calculated at each step  
188 incorporated any previous digesta volume change (with the addition of enzyme/buffer solution and  
189 previous sample removal) over the course of the experiment.

190 *2.10 Statistical Analysis* - Data was analyzed using R statistical software<sup>38</sup>. A mixed factorial  
191 design with repeated measures ANOVA was employed to test the fixed factors of meal (between  
192 subject factor with 3 levels consisting of control, metmyoglobin, and ferrous sulfate), digestion  
193 phase (within subject factor with 4 levels i.e. initial, gastric, duodenal, and jejunal), and  
194 meal\*digestion phase, on carotenoid level (dependent variable). The error factor controlled for  
195 within flask variability. A *P* value < 0.05 was considered statistically significant. Post-hoc analysis  
196 was performed using the Bonferroni correction for multiple comparisons to determine statistically  
197 significant interactions within the same meal over multiple digestion phases, and between meals  
198 at the same digestion phase.

### 199 **3. Results**

200 Figure 1 reveals the loss of lutein in the 3 different meals during the course of *in vitro* digestion.  
201 In the absence of enzymes (Figure 1A), approximately 25% of the initial lutein was lost during the  
202 gastric phase regardless of the meal (i.e. control, metmyoglobin, or ferrous sulfate). During the  
203 duodenal and jejunal phases, no additional loss of lutein was observed with lutein alone or lutein  
204 + ferrous sulfate. Only in the presence of metmyoglobin was an additional 20% of lutein lost during  
205 the duodenal phase. Figure 1B reveals that lutein was more robust through the gastric and duodenal  
206 phases of digestion when enzymes were present, with no difference observed between conditions

207 or phases. However, by the jejunal phase, the results mimicked that of the experiment without  
208 enzymes, and a total of 60% of the lutein remained regardless of meal.

209 Figure 2 demonstrates the loss of lycopene during digestion. In the absence of enzymes (Figure  
210 2A), gradual loss of lycopene was observed at each stage, resulting in a 35% overall loss post-  
211 jejunal phase. The presence of metmyoglobin or ferrous sulfate did not significantly alter lycopene  
212 degradation at any digestion stage. In comparison, the experiment with enzymes (Figure 2B)  
213 revealed no change in lycopene levels with lycopene alone or lycopene + ferrous sulfate until the  
214 jejunal phase, where a ~20% loss was observed. In contrast, a more pronounced lycopene loss was  
215 observed after the duodenal and jejunal phases (12% and 19%, respectively) in the presence of  
216 metmyoglobin.

217 The loss of  $\beta$ -carotene during digestion is shown in Figure 3. In the absence of enzymes  
218 (Figure 3A), an immediate 20% post-gastric loss of  $\beta$ -carotene was observed under all meal  
219 conditions, but there was no further significant  $\beta$ -carotene loss when alone or together with ferrous  
220 sulfate during the rest of the experiment. In contrast, an additional 20% of  $\beta$ -carotene was lost in  
221 the presence of metmyoglobin during the duodenal phase. In the presence of enzymes (Figure 3B),  
222 a 25% loss of  $\beta$ -carotene when alone was observed post-gastric phase, but no additional significant  
223 loss was observed through the duodenal and jejunal phases. When ferrous sulfate was added  
224 together with  $\beta$ -carotene, this resulted in a ~40%  $\beta$ -carotene loss during the gastric phase, but no  
225 further loss through the duodenal and jejunal phases was observed. By comparison, when  
226 metmyoglobin was added to  $\beta$ -carotene, a 50%  $\beta$ -carotene loss was observed during the gastric  
227 phase and a further 30% loss from initial  $\beta$ -carotene level during the duodenal phase of digestion.

228 No significant change in % *cis*-isomers was observed between the starting material and the  
229 jejunal phase for any of the 3 carotenoids tested. Thus, the bars in Figures 1, 2, and 3 represent  
230 total carotenoid (i.e. the summation of all-*trans* + *cis* isomers) at each stage.

#### 231 **4. Discussion**

232 The relative susceptibility of these three carotenoids to loss under various conditions of  
233 digestion presents some intriguing results. We begin by comparing the behavior of carotenoids  
234 alone digested without enzymes, as compared to carotenoids digested with enzymes (solid bars, A  
235 vs. B in Figures 1, 2, and 3). Our experiments clearly reveal that a greater percentage of lutein and  
236 lycopene survived the gastric and duodenal phases of digestion in the presence of enzymes, as  
237 compared to the experiments without enzymes. In the experiments with enzymes, lipases and bile  
238 salts work in concert to reduce the size of emulsified lipid droplets and create mixed micelles  
239 during the duodenal phase.<sup>39</sup> In contrast, the digestion experiments without enzymes were  
240 performed to better understand the impact of chemical conditions alone on carotenoid degradation.  
241 Thus, these experiments contained the emulsified test meal throughout each phase. Studies on the  
242 stability of  $\beta$ -carotene<sup>40,41</sup> and lutein<sup>40</sup> emulsions have demonstrated that carotenoid stability is  
243 directly proportional to lipid droplet size. Consequently, we expected the larger emulsified lipid  
244 droplets produced in the experiments without enzyme to be more protective than small particle  
245 micelles in the experiments with enzymes. Therefore, it was initially quite surprising to observe  
246 no difference in  $\beta$ -carotene degradation, and a more rapid lutein and lycopene degradation in the  
247 experiments without enzymes. However, the enzyme experiments also contained various proteins,  
248 such as amylase during the initial sample treatment, pepsin added during the gastric phase, and the  
249 porcine pancreatin preparation added during the duodenal phase. Previous *in vitro* studies have  
250 demonstrated that certain whey proteins further protect  $\beta$ -carotene<sup>41</sup> and lutein<sup>40</sup> from degradation

251 in emulsification model systems, as these proteins are believed to rest at the interface between the  
252 lipid droplet and the aqueous solution.<sup>41</sup> Similarly, a whey-protein encapsulated lycopene has been  
253 shown to successfully deliver lycopene in humans, presumably via at least partial protection of  
254 lycopene through gastro-intestinal conditions.<sup>42</sup> In short, our results suggest that protein solutions  
255 confer additional protection to lutein and lycopene against chemical oxidation during the gastric  
256 and duodenal phases of digestion, but any advantage gleaned from protein is lost during the jejunal  
257 phase (likely due to carotenoid transfer to and retention in less-protective micelles).

258  $\beta$ -carotene was equally susceptible to degradation, regardless of the absence or presence of  
259 enzymes (solid bars, Figure 3A vs. 3B). It should be noted that these experiments with  $\beta$ -carotene  
260 were repeated (with the repeat experiments also performed in triplicate), with the same average  $\beta$ -  
261 carotene level and variability observed each time. Previous research has demonstrated that the %  
262 carotenoid transferred from emulsion lipid droplet to micelle is inversely proportional to  
263 carotenoid hydrophobicity, with lutein >  $\beta$ -carotene > lycopene.<sup>39</sup> Thus, we would expect that any  
264 “emulsion effect” would more dramatically impact lutein as compared to  $\beta$ -carotene. Similarly,  
265 we would anticipate that protein confers the same type of protection to  $\beta$ -carotene as compared to  
266 lutein or lycopene.<sup>41</sup> Thus, it is not clear why no difference in  $\beta$ -carotene was observed regardless  
267 of the absence or presence of enzymes.

268 Of those tested, lycopene alone was found to be the most stable carotenoid through the  
269 entire digestive process with enzymes, with a 20% loss overall. In contrast, 40% of lutein alone  
270 and  $\beta$ -carotene alone were lost during their respective digestions with enzymes. The lycopene and  
271  $\beta$ -carotene results are in good agreement with Blanquet-Diot et al., who reported a 20% and ~30%  
272 loss, respectively using a dynamic *in vitro* digestion model.<sup>43</sup> In contrast, Courraud et al., reported  
273 a 50% loss of  $\beta$ -carotene after the gastric phase, and a further 20% loss during the intestinal

274 phase<sup>44</sup>. These differences in  $\beta$ -carotene stability are likely due to Courraud et al.'s direct  
275 application of  $\beta$ -carotene dissolved in acetone to the mimicked digesta, and a much lower pH of 2  
276 used during a prolonged gastric phase.<sup>44</sup> Lutein loss was similar to that reported by Courraud et al.  
277 in raw spinach.<sup>44</sup> Other studies by Blanquet-Diot et al., and Chitchumroonchokchai et al., observed  
278 no change in lutein levels over the course of *in vitro* digestion<sup>43,45</sup>. It is likely that the use of a  
279 tomato food matrix<sup>43</sup> and the absence of a jejunal digestion phase<sup>45</sup> are the source of this  
280 divergence.

281         Regardless of the presence or absence of enzymes, ferrous sulfate had no additional impact  
282 on carotenoid loss during the experiments (white bars, Figures 1, 2, and 3). Previous work by our  
283 group has demonstrated that in a gastric-like micellar model, increasing levels of ferrous iron  
284 relative to  $\beta$ -carotene (0-10 equivalents) result in increasing levels of  $\beta$ -carotene oxidation, as  
285 compared to no iron. However, *excess* levels of ferrous iron relative to  $\beta$ -carotene (50 equivalents)  
286 resulted in an initial oxidative loss of  $\beta$ -carotene over 5-10 min, but surprisingly less  $\beta$ -carotene  
287 oxidation through 120 min. It was postulated that when ferrous iron is present in excess, various  
288 perferryl and ferryl intermediates are formed, which further react with ferrous iron to produce high  
289 levels of a diferric ether product, which is inert.<sup>15</sup> The dose of ferrous sulfate and carotenoid used  
290 in this study were calculated to reproduce a plausible scenario of a single iron tablet prescribed as  
291 a supplement (delivering 80 mg of divalent iron) consumed with a meal containing 20 mg  
292 carotenoid. Under these conditions, the iron to carotenoid equivalent is 37 for meals containing  
293 lycopene or  $\beta$ -carotene + ferrous sulfate, and 39 for meals containing lutein + ferrous sulfate. Based  
294 upon our results, we speculate that the ferrous sulfate levels were sufficiently high to result in the  
295 formation of inert diferric ether, resulting in no additional carotenoid oxidation in these groups. It

296 is also possible that the gelatin coating and maltodextrin excipient of the iron tablet exerted a  
297 protective carotenoid effect during the initial phase of digestion.<sup>46</sup>

298         The concentration of metmyoglobin used in our studies (40  $\mu$ M) is reflective of the quantity  
299 expected from the consumption of a 255 g (9 oz.) beef steak (estimated to deliver 30-50  $\mu$ M  
300 metmyoglobin).<sup>47-49</sup> In the *in vitro* experiments with enzymes, metmyoglobin had no impact on  
301 lutein stability (striped bars, Figure 1B), but a more pronounced impact on carotene stability  
302 (striped bars, Figures 2B and 3B), especially that of  $\beta$ -carotene. These results are in good  
303 agreement with similar work observing the capacity of various carotenoids to inhibit  
304 metmyoglobin-induced peroxidation of linoleic acid in an emulsion model.<sup>16</sup> Inhibitory concentrations  
305 at 50% of maximal linoleic acid peroxidation (IC<sub>50</sub>) values were measured, and were shown to  
306 follow the order of  $\beta$ -carotene > lycopene > xanthophylls derived from bacteria.<sup>16</sup> In other words,  
307 a larger concentration of  $\beta$ -carotene was needed to protect linoleic acid relative to the other  
308 carotenoids. Further measures of the peroxide kinetics suggested that this difference may lie in the  
309 fact that carotenes sit in the center of the lipid droplet, and thus their only mechanism of defense  
310 is to halt further fatty acid peroxide propagation. In contrast, bacterial xanthophylls sit at the lipid-  
311 water interface and likely inhibit peroxidation initiation altogether.<sup>16</sup> It is not clear why  $\beta$ -carotene  
312 was more sensitive than lycopene to metmyoglobin-induced oxidation. It is possible that at the  
313 concentrations of lycopene used, lycopene microcrystals may have formed in the digesta mixture<sup>50</sup>,  
314 which may have conferred physico-chemical protection, while  $\beta$ -carotene likely remained  
315 dissolved.

316         We intentionally used un-stripped sunflower oil in all of our experiments to create a  
317 realistic liquid beverage that would be safe for use in a human clinical study. However, the  
318 endogenous tocopherols and tocotrienols present likely protected carotenoids and reduced losses

319 due to oxidation, as studies have previously demonstrated that alpha-tocopherol protects  
320 lycopene<sup>51,52</sup>,  $\beta$ -carotene<sup>53</sup>, and lutein<sup>40</sup> from various forms of oxidation in emulsion systems.

321 It should be noted that under typical digestive conditions (i.e. with enzymes), levels of lutein  
322 alone and lycopene alone were not significantly different from the initial meal through the  
323 duodenal phase. Immunohistochemical staining demonstrates the presence of apical membrane  
324 transporters SR-B1 and CD-36 in the duodenum and jejunum of humans<sup>54</sup>, with Western blotting  
325 showing significantly higher SR-B1 protein expression in the duodenum<sup>55</sup> and higher expression  
326 of CD-36 in the jejunum<sup>56</sup> of rodents. Thus, we anticipate a fair portion of lutein and lycopene to  
327 be absorbed in the duodenum (before degradation), and any resulting loss in the jejunum would  
328 probably have a limited impact on overall bioavailability. However, our results suggest that partial  
329 degradation of  $\beta$ -carotene alone under these types of meal conditions may ultimately reduce the  
330 quantity of  $\beta$ -carotene that is bioavailable.

331 We noted no significant change from the initial % *cis* isomers to the % *cis* isomers remaining  
332 in jejunal phase. These results are in good agreement with previous studies using static *in vitro*  
333 digestion, which have demonstrated that the % *cis* isomer contribution to total carotenoid content  
334 is well maintained for lycopene<sup>57,58</sup>, lutein<sup>59</sup>, and  $\beta$ -carotene.<sup>60</sup> These results are further  
335 corroborated with human studies on gastric digesta for lycopene and  $\beta$ -carotene.<sup>61</sup> The same  
336 human study also investigated duodenal digesta, and found no change in lycopene but a slight  
337 increase in % *cis*  $\beta$ -carotene (relative to starting % *cis*  $\beta$ -carotene).<sup>61</sup> We would attribute this  
338 disparity to the dynamic nature of absorption in humans, and transporter selectivity for all-*trans*-  
339  $\beta$ -carotene.<sup>62</sup>

340 While our primary objective was to understand carotenoid loss during digestion, we also  
341 anticipated observing carotenoid degradation products in our samples. Indeed, the extraction and



342 HPLC-PDA methods employed for the analysis of  $\beta$ -carotene- and lycopene-meal digesta would  
343 have revealed the presence of  $\beta$ -apo-carotenals and apo-lycopenals, respectively, if they had been  
344 present in sufficiently high quantities for PDA detection. Furthermore, previous work by our group  
345 has demonstrated that under certain mimicked conditions of gastric digestion and in the presence  
346 of metmyoglobin,  $\beta$ -apo-carotenals can be observed by PDA.<sup>15</sup> However, no oxidation products  
347 were observed in any of the samples in this study. This discrepancy with previous work may be  
348 due to the previous use of non-biological surfactants (Tween 20 and Brij 35) to create micelle-like  
349 conditions.<sup>15</sup> Similarly, aldehydes have the capacity to react irreversibly with proteins to form  
350 Schiff bases<sup>63</sup> and thus it is possible that aldehyde products were formed in our study, but were  
351 not able to be extracted due to protein sequestration. While apo-carotenaldehydes were not  
352 observed, the loss of lutein and lycopene in the jejunal phase, and the immediate loss of  $\beta$ -carotene  
353 in the gastric phase, indicate that at least some derivatives were created. Further work is needed to  
354 identify these products, and to determine if they are absorbed, and/or if they might have biological  
355 effects in the jejunum or colon.

356 In conclusion, some of the chemical factors that reduce carotenoid stability in an *in vitro*  
357 emulsion model are counteracted in the presence of digestive enzymes. In the presence of enzymes,  
358 lutein and lycopene were robust to the effects of the digestive process throughout the gastric and  
359 duodenal phases, with the most pronounced losses occurring during the jejunal phase.  $\beta$ -carotene  
360 was most susceptible to degradation during the gastric phase, but no further loss was observed.  
361 Our data suggests that a commonly prescribed dose of supplemental iron consumed with a  
362 carotenoid-containing meal does not have an impact on carotenoid stability. However, co-  
363 consumption of red meat with lycopene- or  $\beta$ -carotene-containing foods is likely to reduce the  
364 quantity of carotenoid available for absorption, and may generate carotenoid oxidation products.

365 Further work is needed to determine which products are produced, and whether they have  
366 biologically relevant actions.

367

368 **Conflict of Interest**

369 There are no conflicts of interest to declare.

370

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379

380 **Abbreviations:**

381 DCM dichloromethane

382 HPLC high-performance liquid chromatography

383 LC-MS liquid chromatography-mass spectrometry

384 MeOH methanol

385 MTBE methyl *tert*-butyl ether

386 PDA photodiode array detection

387

388 **References**

- 389 1 E. Giovannucci, Tomatoes, tomato-based products, lycopene, and cancer: review of the  
390 epidemiologic literature, *J. Natl. Cancer Inst.*, 1999, **91**, 317–331.
- 391 2 H. D. Sesso and J. M. Gaziano, Heart and vascular diseases, in *Carotenoids in Health and*  
392 *Disease*, Marcel Dekker, New York, 2004, pp. 491–502.
- 393 3 B. Halliwell, Oxidative stress and cancer: have we moved forward?, *Biochem. J.*, 2007,  
394 **401**, 1–11.
- 395 4 N. R. Madamanchi, A. Vendrov and M. S. Runge, Oxidative stress and vascular disease,  
396 *Arterioscler. Thromb. Vasc. Biol.*, 2005, **25**, 29–38.
- 397 5 M. F. Beal, Aging, energy, and oxidative stress in neurodegenerative diseases, *Ann.*  
398 *Neurol.*, 1995, **38**, 357–366.
- 399 6 N. I. Krinsky, Carotenoids and cancer in animal models, *J. Nutr.*, 1989, **119**, 123–126.
- 400 7 W. Stahl and H. Sies, Lycopene: A biologically important carotenoid for humans?, *Arch.*  
401 *Biochem. Biophys.*, 1996, **336**, 1–9.
- 402 8 A. Cantrell, D. J. McGarvey, T. George Truscott, F. Rancan and F. Böhm, Singlet oxygen  
403 quenching by dietary carotenoids in a model membrane environment, *Arch. Biochem.*  
404 *Biophys.*, 2003, **412**, 47–54.
- 405 9 J. E. Romanchik, D. W. Morel and E. H. Harrison, Distributions of carotenoids and  $\alpha$ -  
406 tocopherol among lipoproteins do not change when human plasma is incubated in vitro, *J.*  
407 *Nutr.*, 1995, **125**, 2610–2617.
- 408 10 S. Stanner, J. Hughes, C. Kelly and J. Buttriss, A review of the epidemiological evidence  
409 for the “antioxidant hypothesis,” *Public Health Nutr.*, 2004, **7**, 407–422.

- 410 11 C. A. Rice-Evans, J. Sampson, P. M. Bramley and D. E. Holloway, Why do we expect  
411 carotenoids to be antioxidants in vivo?, *Free Radic. Res.*, 1997, **26**, 381–398.
- 412 12 S. A. R. Paiva and R. M. Russell,  $\beta$ -Carotene and other carotenoids as antioxidants, *J. Am.*  
413 *Coll. Nutr.*, 1999, **18**, 426–433.
- 414 13 B. Halliwell, K. Zhao and M. Whiteman, The gastrointestinal tract: A major site of  
415 antioxidant action?, *Free Radic. Res.*, 2000, **33**, 819–830.
- 416 14 J. Kanner and T. Lapidot, The stomach as a bioreactor: dietary lipid peroxidation in the  
417 gastric fluid and the effects of plant-derived antioxidants, *Free Radic. Biol. Med.*, 2001, **31**,  
418 1388–1395.
- 419 15 C. Sy, O. Dangles, P. Borel and C. Caris-Veyrat, Iron-induced oxidation of (all-E)- $\beta$ -  
420 carotene under model gastric conditions: kinetics, products, and mechanism, *Free Radic.*  
421 *Biol. Med.*, 2013, **63**, 195–206.
- 422 16 C. Sy, C. Caris-Veyrat, C. Dufour, M. Boutaleb, P. Borel and O. Dangles, Inhibition of  
423 iron-induced lipid peroxidation by newly identified bacterial carotenoids in model gastric  
424 conditions: comparison with common carotenoids, *Food Funct.*, 2013, **4**, 698–712.
- 425 17 P. Goupy, E. Reynaud, O. Dangles and C. Caris-Veyrat, Antioxidant activity of (all-E)-  
426 lycopene and synthetic apo-lycopenoids in a chemical model of oxidative stress in the  
427 gastro-intestinal tract, *N. Journal Chem.*, 2012, **36**, 575–587.
- 428 18 E. Vulcain, P. Goupy, C. Caris-Veyrat and O. Dangles, Inhibition of the metmyoglobin-  
429 induced peroxidation of linoleic acid by dietary antioxidants: Action in the aqueous vs. lipid  
430 phase, *Free Radic. Res.*, 2005, **39**, 547–563.

- 431 19 S. Kiokias, C. Dimakou and V. Oreopoulou, Activity of natural carotenoid preparations  
432 against the autoxidative deterioration of sunflower oil-in-water emulsions, *Food Chem.*,  
433 2009, **114**, 1278–1284.
- 434 20 S. Kiokias and T. Varzakas, Activity of flavonoids and  $\beta$ -carotene during the auto-oxidative  
435 deterioration of model food oil-in water emulsions, *Food Chem.*, 2014, **150**, 280–286.
- 436 21 E. Reboul, M. Richelle, E. Perrot, C. Desmoulins-Malezet, V. Pirisi and P. Borel,  
437 Bioaccessibility of carotenoids and vitamin E from their main dietary sources, *J. Agric.*  
438 *Food Chem.*, 2006, **54**, 8749–8755.
- 439 22 K. H. van Het Hof, C. E. West, J. A. Weststrate and J. G. Hautvast, Dietary factors that  
440 affect the bioavailability of carotenoids, *J. Nutr.*, 2000, **130**, 503–506.
- 441 23 P. Borel, D. Preveraud and C. Desmarchelier, Bioavailability of vitamin E in humans: An  
442 update, *Nutr. Rev.*, 2013, **71**, 319–331.
- 443 24 J. Dyerberg, P. Madsen, J. M. Møller, I. Aardestrup and E. B. Schmidt, Bioavailability of  
444 marine n-3 fatty acid formulations, *Prostaglandins Leukot. Essent. Fatty Acids*, 2010, **83**,  
445 137–141.
- 446 25 M. Carail and C. Caris-Veyrat, Carotenoid oxidation products: From villain to saviour?,  
447 *Pure Appl. Chem.*, 2006, **78**, 1493–1503.
- 448 26 J. W. Erdman, N. A. Ford and B. L. Lindshield, Are the health attributes of lycopene related  
449 to its antioxidant function?, *Arch. Biochem. Biophys.*, 2009, **483**, 229–235.
- 450 27 A. Eroglu, D. P. Hruszkewycz, C. dela Sena, S. Narayanasamy, K. M. Riedl, R. E. Kopec,  
451 S. J. Schwartz, R. W. Curley Jr and E. H. Harrison, Naturally occurring eccentric cleavage  
452 products of provitamin A  $\beta$ -carotene function as antagonists of retinoic acid receptors, *J.*  
453 *Biol. Chem.*, 2012, **287**, 15886–15895.

- 454 28 R. E. Kopec, K. M. Riedl, E. H. Harrison, R. W. Curley Jr, D. P. Hruszkewycz, S. K.  
455 Clinton and S. J. Schwartz, Identification and quantification of apo-lycopenals in fruits,  
456 vegetables, and human plasma, *J. Agric. Food Chem.*, 2010, **58**, 3290–3296.
- 457 29 F. Khachik, C. J. Spangler, J. C. Smith, L. M. Canfield, A. Steck and H. Pfander,  
458 Identification, quantification, and relative concentrations of carotenoids and their  
459 metabolites in human milk and serum, *Anal. Chem.*, 1997, **69**, 1873–1881.
- 460 30 G. Serratrice, H. Boukhalifa, C. Béguin, P. Baret, C. Caris and J.-L. Pierre, O-TRENTOX, a  
461 New Tripodal Iron Chelator Based on 8-Hydroxyquinoline Subunits: Thermodynamic and  
462 Kinetic Studies, *Inorg. Chem.*, 1997, **36**, 3898–3910.
- 463 31 W. J. Bowen, The absorption spectra and extinction coefficients of myoglobin, *J. Biol.*  
464 *Chem.*, 1949, **179**, 235–245.
- 465 32 D. A. Garrett, M. L. Failla and R. J. Sarama, Development of an in vitro digestion method  
466 to assess carotenoid bioavailability from meals, *J. Agric. Food Chem.*, 1999, **47**, 4301–  
467 4309.
- 468 33 B. Gleize, F. Tourniaire, L. Depezay, R. Bott, M. Nowicki, L. Albino, D. Lairon, E. Kesse-  
469 Guyot, P. Galan, S. Hercberg and P. Borel, Effect of type of TAG fatty acids on lutein and  
470 zeaxanthin bioavailability, *Br. J. Nutr.*, 2013, **110**, 1–10.
- 471 34 G. A. Garzón, C.-E. Narváez-Cuenca, R. E. Kopec, A. M. Barry, K. M. Riedl and S. J.  
472 Schwartz, Determination of carotenoids, total phenolic content, and antioxidant activity of  
473 Arazá (*Eugenia stipitata* McVaugh), an Amazonian fruit, *J. Agric. Food Chem.*, 2012, **60**,  
474 4709–4717.

- 475 35 L. K. Henry, G. L. Catignani and S. J. Schwartz, Oxidative degradation kinetics of  
476 lycopene, lutein, and 9-cis and all-trans  $\beta$ -carotene, *J. Am. Oil Chem. Soc.*, 1998, **75**, 823–  
477 829.
- 478 36 K. Fröhlich, J. Conrad, A. Schmid, D. E. Breithaupt and V. Böhm, Isolation and structural  
479 elucidation of different geometrical isomers of lycopene, *Int. J. Vitam. Nutr. Res.*, 2007, **77**,  
480 369–375.
- 481 37 V. Böhm, Use of column temperature to optimize carotenoid isomer separation by C30 high  
482 performance liquid chromatography, *J. Sep. Science*, 2001, **24**, 955–959.
- 483 38 R Core Team, R: A language and environment for statistical computing. R Foundation for  
484 Statistical Computing, Vienna, Austria., 2013. . Available at: <http://www.R-project.org/>.
- 485 39 V. Tyssandier, B. Lyan and P. Borel, Main factors governing the transfer of carotenoids  
486 from emulsion lipid droplets to micelles, *BBA - Mol. Cell Biol. L.*, 2001, **1533**, 285–292.
- 487 40 K. Frede, A. Henze, M. Khalil, S. Baldermann, F. J. Schweigert and H. Rawel, Stability and  
488 cellular uptake of lutein-loaded emulsions, *J. Funct. Foods*, 2014, **8**, 118–127.
- 489 41 L. Cornacchia and Y. H. Roos, Stability of  $\beta$ -carotene in protein-stabilized oil-in-water  
490 delivery systems, *J. Agric. Food Chem.*, 2011, **59**, 7013–7020.
- 491 42 M. Richelle, K. Bortlik, S. Liardet, C. Hager, P. Lambelet, M. Baur, L. A. Applegate and E.  
492 A. Offord, A food-based formulation provides lycopene with the same bioavailability to  
493 humans as that from tomato paste, *J. Nutr.*, 2002, **132**, 404–408.
- 494 43 S. Blanquet-Diot, M. Soufi, M. Rambeau, E. Rock and M. Alric, Digestive stability of  
495 xanthophylls exceeds that of carotenes as studied in a dynamic in vitro gastrointestinal  
496 system, *J. Nutr.*, 2009, **139**, 876–883.



- 497 44 J. Courraud, J. Berger, J.-P. Cristol and S. Avallone, Stability and bioaccessibility of  
498 different forms of carotenoids and vitamin A during in vitro digestion, *Food Chem.*, 2013,  
499 **136**, 871–877.
- 500 45 C. Chitchumroonchokchai, S. J. Schwartz and M. L. Failla, Assessment of lutein  
501 bioavailability from meals and a supplement using simulated digestion and caco-2 human  
502 intestinal cells, *J. Nutr.*, 2004, **134**, 2280–2286.
- 503 46 D. J. Cox, D. R. Kearney, S. T. Kirksey Jr and M. J. Taylor, Oil-in-water dispersions of  $\beta$ -  
504 carotene and other carotenoids stable against oxidation prepared from water-dispersible  
505 beadlets having high concentrations of carotenoid, US6007856 A, 1999.
- 506 47 J. D. Love and A. M. Pearson, Metmyoglobin and nonheme iron as prooxidants in cooked  
507 meat, *J. Agric. Food Chem.*, 1974, **22**, 1032–1034.
- 508 48 H. B. Craig, T. N. Blumer, W. W. G. Smart and M. B. Wise, Evaluation of hemoglobin,  
509 myoglobin, blood oxygen content and organoleptic qualities of beef from steers fed grain  
510 on pasture or cut forage and grain in drylot, *J. Animal Sci.*, 1966, **25**, 1128–1132.
- 511 49 K. Sato and G. R. Hegarty, Warmed-over flavor in cooked meats, *J. Food Sci.*, 1971, **36**,  
512 1098–1102.
- 513 50 D. J. McClements, Crystals and crystallization in oil-in-water emulsions: Implications for  
514 emulsion-based delivery systems, *Adv. Colloid Interface Sci.*, 2012, **174**, 1–30.
- 515 51 R. Bou, C. Boon, A. Kweku, D. Hidalgo and E. A. Decker, Effect of different antioxidants  
516 on lycopene degradation in oil-in-water emulsions, *Eur. J. Lipid Sci. Technol.*, 2011, **113**,  
517 724–729.
- 518 52 C. S. Boon, Z. Xu, X. Yue, D. J. McClements, J. Weiss and E. A. Decker, Factors affecting  
519 lycopene oxidation in oil-in-water emulsions, *J. Agric. Food Chem.*, 2008, **56**, 1408–1414.

- 520 53 M. Heinonen, K. Haila, A.-M. Lampi and V. Piironen, Inhibition of oxidation in 10% oil-  
521 in-water emulsions by  $\beta$ -carotene with  $\alpha$ - and  $\gamma$ -tocopherols, *J. Amer. Oil Chem. Soc.*, 1997,  
522 **74**, 1047.
- 523 54 M. V. Lobo, L. Huerta, N. Ruiz-Velasco, E. Teixeira, P. de la Cueva, A. Celdrán, A.  
524 Martín-Hidalgo, M. A. Vega and R. Bragado, Localization of the lipid receptors CD36 and  
525 CLA-1/SR-BI in the human gastrointestinal tract: towards the identification of receptors  
526 mediating the intestinal absorption of dietary lipids, *J. Histochem. Cytochem.*, 2001, **49**,  
527 1253–1260.
- 528 55 S. F. Cai, R. J. Kirby, P. N. Howles and D. Y. Hui, Differentiation-dependent expression  
529 and localization of the class B type I scavenger receptor in intestine, *J. Lipid Res.*, 2001, **42**,  
530 902–909.
- 531 56 M. Chen, Y. Yang, E. Braunstein, K. E. Georgeson and C. M. Harmon, Gut expression and  
532 regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes, *Am. J.*  
533 *Physiol. Endocrinol. Metab.*, 2001, **281**, E916–E923.
- 534 57 M. L. Failla, C. Chitchumroonchokchai and B. K. Ishida, In vitro micellarization and  
535 intestinal cell uptake of cis isomers of lycopene exceed those of all-trans lycopene, *J. Nutr.*,  
536 2008, **138**, 482–486.
- 537 58 M. Richelle, B. Sanchez, I. Tavazzi, P. Lambelet, K. Bortlik and G. Williamson, Lycopene  
538 isomerisation takes place within enterocytes during absorption in human subjects, *Br. J.*  
539 *Nutr.*, 2010, **103**, 1800–1807.
- 540 59 F. Granado-Lorencio, B. Olmedilla-Alonso, C. Herrero-Barbudo, B. Pérez-Sacristan, I.  
541 Blanco-Navarro and S. Blazquez-García, Comparative in vitro bioaccessibility of

542 carotenoids from relevant contributors to carotenoid intake, *J. Agric. Food Chem.*, 2007,  
543 **55**, 6387–6394.

544 60 M. G. Ferruzzi, J. L. Lumpkin, S. J. Schwartz and M. Failla, Digestive stability,  
545 micellarization, and uptake of  $\beta$ -carotene isomers by Caco-2 human intestinal cells, *J.*  
546 *Agric. Food Chem.*, 2006, **54**, 2780–2785.

547 61 V. Tyssandier, E. Reboul, J.-F. Dumas, C. Bouteloup-Demange, M. Armand, J. Marcand,  
548 M. Sallas and P. Borel, Processing of vegetable-borne carotenoids in the human stomach  
549 and duodenum, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2003, **284**, G913-923.

550 62 A. During, M. M. Hussain, D. W. Morel and E. H. Harrison, Carotenoid uptake and  
551 secretion by CaCo-2 cells:  $\beta$ -carotene isomer selectivity and carotenoid interactions, *J.*  
552 *Lipid Res*, 2002, **43**, 1086–1095.

553 63 D. J. Tuma, T. Hoffman and M. F. Sorrell, The chemistry of acetaldehyde-protein adducts,  
554 *Alcohol Alcohol. Suppl.*, 1991, **1**, 271–276.

555 64 M. Armand, P. Borel, C. Dubois, M. Senft, J. Peyrot, J. Salducci, H. Lafont and D. Lairon,  
556 Characterization of emulsions and lipolysis of dietary lipids in the human stomach, *Am. J.*  
557 *Physiol.*, 1994, **266**, G372-381.

558

559 **Table 1.** Composition of the test meals

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Water	90 g
Sucrose	9 g
Phospholipid containing powder <sup>a, b</sup>	150 mg
Sunflower oil	3 g
Carotenoid-oil preparation <sup>b</sup>	0.3 g

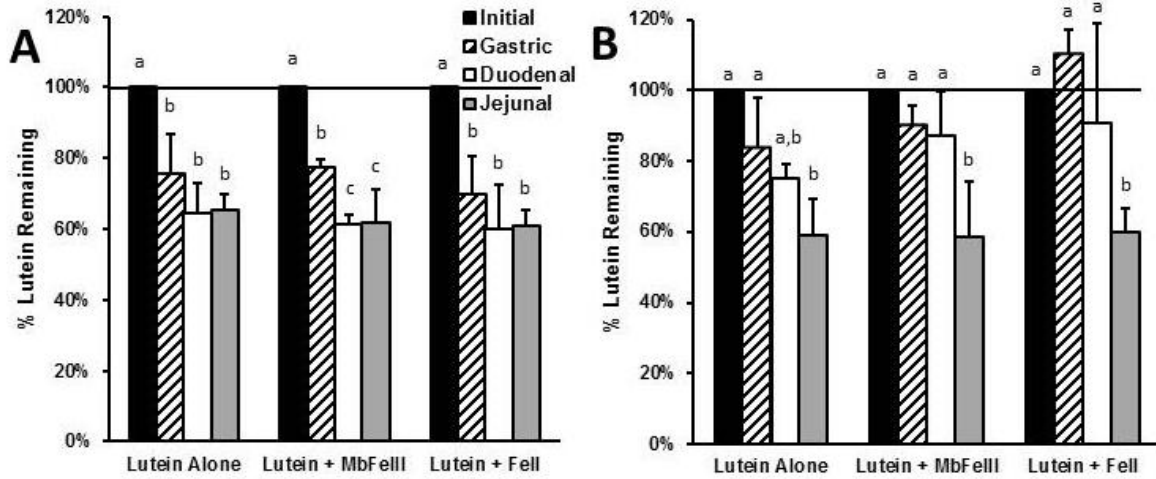
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560 a) Delivering 75 mg of phospholipid derived from egg, to match the 1:40 ratio of  
561 phospholipid to lipid representative of a western diet<sup>64</sup>.

562 b) See Materials and Methods for additional details.

563

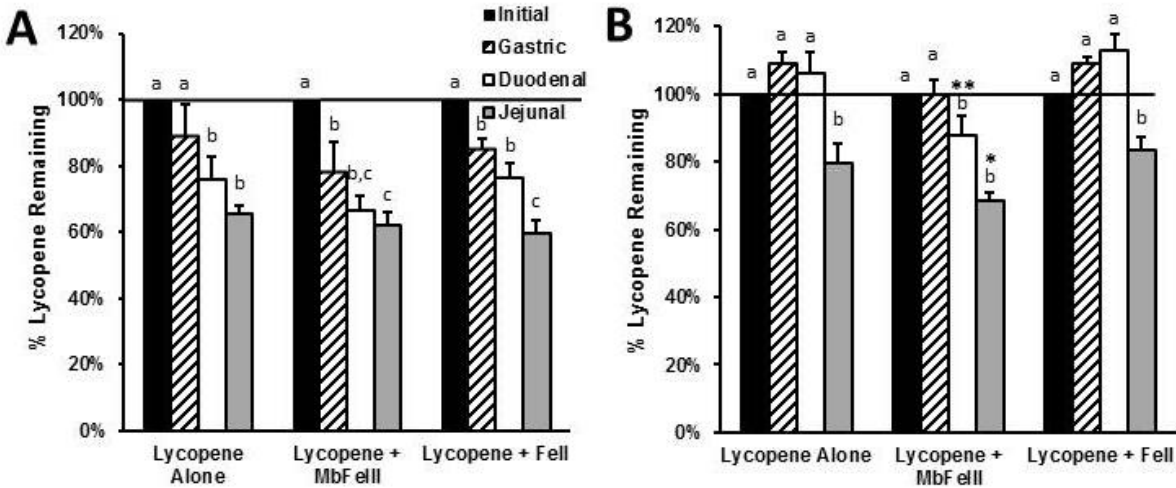
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565

566 **Figure 1.** Loss of initial lutein through the gastric, duodenal, and jejunal phases of digestion in  
 567 the absence (A) and presence (B) of digestive enzymes. Bars represent average % lutein  $\pm$   
 568 standard deviation (n=3 for each meal). Different letters indicate statistically significant  
 569 differences within the same meal at different digestion phases ( $P < 0.05$ ). There was no  
 570 significant difference between meal types at any of the digestion phases ( $P < 0.05$ ).

571



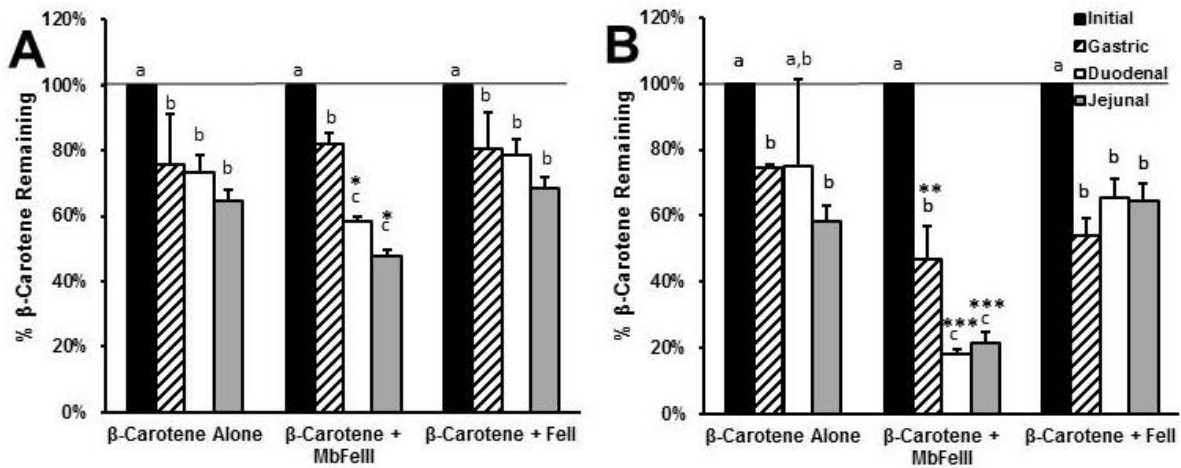
572

573 **Figure 2.** Loss of initial lycopene through the gastric, duodenal, and jejunal phases of digestion  
 574 in the absence (A) and presence (B) of digestive enzymes. Bars represent average % lycopene ±  
 575 standard deviation (n=3 for each meal). Different letters indicate statistically significant  
 576 differences within the same meal at different digestion phases ( $P < 0.05$ ). Statistically significant  
 577 differences between meal types in the same digestion phase are noted with an asterisk, described  
 578 below.

579 \*Significantly different from Lycopene + FeII after the same digestion phase ( $P < 0.001$ )

580 \*\*Significantly different from Lycopene Alone and Lycopene + FeII after the same digestion  
 581 phase ( $P < 0.0001$ )

582



583

584 **Figure 3.** Loss of initial  $\beta$ -carotene through the gastric, duodenal, and jejunal phases of digestion  
 585 in the absence (A) and presence (B) of digestive enzymes. Bars represent average %  $\beta$ -carotene  $\pm$   
 586 standard deviation (n=3 for each meal). Different letters indicate statistically significant  
 587 differences within the same meal at different digestion phases ( $P < 0.05$ ). Statistically significant  
 588 differences between meal types in the same digestion phase are noted with an asterisk, described  
 589 below.

590 \*Significantly different from  $\beta$ -Carotene + FeII after the same digestion phase ( $P < 0.005$ )

591 \*\*Significantly different from  $\beta$ -Carotene Alone after the same digestion phase ( $P = 0.006$ )

592 \*\*\*Significantly different from  $\beta$ -Carotene Alone and  $\beta$ -Carotene + FeII after the same digestion  
 593 phase ( $P < 0.0001$ )

594