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Lycopene bioavailability is associated with a combination of genetic variants

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Running head: Lycopene bioavailability

- 1 **Abbreviations:** ABCB1 (ATP-binding cassette, sub-family B (MDR/TAP), member 1);
- 2 AUC (area under the curve); BCO1 (β -carotene 15,15'-oxygenase-1); BCO2 (β -carotene

3 9,10'-oxygenase-2); CD36 (cluster determinant 36); CM (chylomicron); ELOVL2 (ELOVL
4 fatty acid elongase 2); HPLC (high performance liquid chromatography); ISX (intestine
5 specific homeobox); L-FABP (liver-fatty acid binding protein); LYC (lycopene); MTTP
6 (microsomal triglyceride transfer protein); PLS (partial least squares); SCARB1 (scavenger
7 receptor class B, member 1); SNPs (single nucleotide polymorphisms); SOD2 (superoxide
8 dismutase 2, mitochondrial); SR-BI (scavenger receptor class B type I); VIP (variable
9 importance in the projection).

10

11 This clinical trial is registered at <http://www.clinicaltrials.gov/ct2/>. The ID number is
12 NCT02100774.

13

14

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16

17

18

19 **Footnote:** chylomicron (CM), Lycopene (LYC), single nucleotide polymorphism (SNP),
20 partial least square regression (PLS)

21 **Abstract**

22 **Background:** The intake of tomatoes and tomato products, which constitute the main dietary
23 source of the red pigment lycopene (LYC), has been associated with a reduced risk of prostate
24 cancer and cardiovascular disease, suggesting a protective role of this carotenoid. However,
25 LYC bioavailability displays high interindividual variability. This variability may lead to
26 varying biological effects following LYC consumption. **Objectives:** Based on recent results
27 obtained with two other carotenoids, we assumed that this variability was due, at least in part,
28 to several single nucleotide polymorphisms (SNPs) in genes involved in LYC and lipid
29 metabolism. Thus, we aimed at identifying a combination of SNPs significantly associated
30 with the variability in LYC bioavailability.

31 **Design:** In a postprandial study, 33 healthy male volunteers consumed a test meal containing
32 100 g tomato puree, which provided 9.7 mg all-trans LYC. LYC concentrations were
33 measured in plasma chylomicrons (CM) isolated at regular time intervals over 8 h post-
34 prandially. 1885 SNPs in 49 candidate genes, i.e. genes assumed to play a role in LYC
35 bioavailability, were selected. Multivariate statistical analysis (partial least squares regression)
36 was used to identify and validate the combination of SNPs most closely associated with
37 postprandial CM LYC response.

38 **Results:** The postprandial CM LYC response to the meal was notably variable with a CV of
39 70%. A significant ($P=0.037$) and validated partial least squares regression model, which
40 included 28 SNPs in 16 genes, explained 72 % of the variance in the postprandial CM LYC
41 response. The postprandial CM LYC response was also positively correlated to fasting plasma
42 LYC concentrations ($r=0.37$, $P<0.05$).

43 **Conclusions:** The ability to respond to LYC is explained, at least partly, by a combination of
44 28 SNPs in 16 genes. Interindividual variability in bioavailability apparently affects the long

45 term blood LYC status, which could ultimately modulate the biological response following
46 LYC supplementation.

47

48 **Keywords:** carotenoid; absorption; single nucleotide polymorphisms; nutrigenetic; genetic
49 polymorphisms

50

51 **Introduction**

52 Lycopene (LYC) is the red pigment found in tomatoes and tomato products. It is the
53 carotenoid found at the highest levels in the blood of Americans and the second one in the
54 blood of Europeans. Its protective role against the development of prostate cancer and
55 cardiovascular diseases has been suggested [1-6] but the mechanisms involved have yet to be
56 fully elucidated. Indeed, although LYC's potential as an antioxidant is well characterized in
57 vitro [7], there is evidence that its biological effects could also be mediated by metabolic
58 products of LYC [8-11], a hypothesis supported by the results of recent molecular studies [11-
59 14].

60 LYC digestion begins in the gastrointestinal lumen where digestive enzymes can
61 modulate its bioaccessibility by facilitating its release from the food matrix to micelles [15-
62 17]. Micelles then carry LYC to the apical side of the enterocyte. LYC uptake is not only
63 passive, as previously thought, [18] but its transport is also facilitated by two membrane
64 proteins, SR-BI (scavenger receptor class B type I) [19], encoded by *SCARB1*, and CD36
65 (cluster determinant 36) [20]. Following uptake, it is possible that a portion of LYC may be
66 metabolized. Indeed, enterocytes contain two enzymes with the potential to cleave LYC. The
67 first one, BCO1 (β -carotene 15,15' oxygenase-1), is a dioxygenase [21] that catalyzes the
68 oxidative cleavage of LYC with an efficiency similar to that of β -carotene [22], and which has
69 been associated with blood LYC status [23]. The second enzyme, BCO2 (β -carotene-9,10'-
70 oxygenase), has been previously suggested to be the main LYC-cleaving enzyme [10].
71 Regardless of possible metabolism, the fraction of parent LYC remaining in the enterocyte is
72 transported within the cell to the site where it is incorporated into chylomicrons (CM). The
73 mechanism of this transport is not known, but proteins involved in intracellular transport of
74 lipids have been suggested to be involved [24]. LYC-containing CM are secreted into the

75 lymph, and then enter the bloodstream. LYC is assumed to stay within these lipoproteins and
76 to follow the fate of CM which is ultimately taken up by the hepatocytes [25].

77 Clinical trials dedicated to the study of LYC bioavailability have reported high
78 interindividual variability in blood and tissue LYC concentration in response to LYC intake
79 [18, 26-30]. Genetic variations between individuals may provide a partial explanation of this
80 phenomenon [31, 32]. Some genetic polymorphisms have been shown to be associated with
81 the variability in fasting blood LYC concentrations [23, 30, 33-36], and yet only one study has
82 attempted to assess the role of genetic polymorphisms involved in the variability in blood
83 LYC concentration following a LYC load [30]. Only two SNPs in *BCOI* have been previously
84 shown to be associated with the variability in LYC bioavailability [30]. While promising, the
85 small number of candidate genes previously investigated calls for more studies to explain the
86 variation in a phenotype that is likely affected by numerous environmental and genetic factors
87 [31]. In fact, we have recently demonstrated that the bioavailability of the carotenoid lutein is
88 a complex phenotype that is not modulated by any single gene, but by the additive effects
89 several gene SNPs [37]. Thus, the aim of this study was to identify the combination of SNPs
90 associated with the variability in LYC bioavailability.

91

92 **Subjects and Methods**

93 *Subject number and characteristics*

94 Thirty five healthy, nonobese, nonsmoking male subjects were recruited for the study.
95 Subjects presented with normal energy consumption, i.e. ≈ 2500 kcal/d and drank $\leq 2\%$
96 alcohol as total energy. They had no history of chronic disease, hyperlipidemia,
97 hyperglycemia, and were not taking any medication that might affect LYC or lipid
98 metabolism (e.g. tetrahydrolipstatin, ezetimibe, phytosterols, cholestyramine, fibrates, etc.)
99 during the month prior to the study or during the study period. Because of the relatively large
100 volume of blood that was drawn during the study, subjects were required to have a blood
101 hemoglobin concentration >1.3 g/L as inclusion criteria. The study was approved by the
102 regional committee on human experimentation (N°2008-A01354-51, Comité de Protection des
103 Personnes Sud Méditerranée I, France). The procedures followed were in accordance with the
104 Helsinki Declaration of 1975 as revised in 1983. The objectives and requirements of the study
105 were fully explained to all participants before beginning the study, and informed written
106 consent was obtained from each subject. Two subjects left the study for personal reasons
107 before participating in the postprandial experiment, leaving 33 subjects whose baseline
108 characteristics are reported in **Table 1**. Note that the fasting plasma LYC concentration of
109 these subjects was relatively high as compared to previous reported data [38] suggesting that
110 these subjects regularly consumed tomatoes and tomato products.

111

112 *DNA preparation and genotyping methods*

113 An average of 25 μg of DNA was isolated from a saliva sample from each subject
114 using the Oragene kit (DNA Genotek Inc., Kanata, ON, Canada) as described in detail
115 previously [39]. DNA concentration and purity were checked by spectrophotometry at 260 nm
116 and 280 nm (Nanodrop ND1000, Thermo Scientific, Villebon sur Yvette, France). All

117 genotyping procedures were carried out by Integragen (Evry, France). Concerning the whole
118 genome genotyping, the procedure was as follows: 200 ng of DNA was hybridized overnight
119 to HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA), allowing the analysis of
120 approximately 7.33×10^5 SNPs per DNA sample. Unhybridized and non-specifically
121 hybridized DNA was washed out. The BeadChips were then stained and scanned on an
122 Illumina iScan. Detailed methods are provided in the Infinium HD Assay Ultra Protocol
123 Guide from Illumina. Concerning the 40 other SNPs (see “Choice of candidate genes”), they
124 were genotyped as previously described [40].

125

126 *Postprandial experiments*

127 Subjects were asked to refrain from consuming LYC-rich foods (tomatoes, tomato
128 products, foods rich in tomato, watermelon) for 48 h before the postprandial clinic visit. In
129 addition, the day prior to the visit, they were asked to eat dinner between 7 and 8 p.m.,
130 without any alcohol intake. They were also asked to abstain from consuming any food or
131 beverage other than water after the dinner and until the clinic visit. After the overnight fast,
132 they arrived at the local Center for Clinical Investigation (la Conception Hospital, Marseille,
133 France) and consumed a test meal including 100 g of tomato puree purchased from a local
134 supermarket providing 9.7 mg all-trans LYC as measured by high performance liquid
135 chromatography (HPLC). The puree also contained around 0.5 mg cis isomers of LYC as
136 evaluated by the relative peak area of all the cis-isomers (4 peaks identified) as compared to
137 the peak area of the all-trans isomer in the HPLC chromatograms. This LYC dose allowed us
138 to deliver about twice the mean dietary intake of LYC in France and a LYC dose close to
139 daily intake in the United states [30, 38]. The remainder of the test meal consisted of semolina
140 (70 g) cooked in 200 mL of hot water, white bread (40 g), egg whites (60 g), peanut oil (50 g),
141 and mineral water (330 mL). The subjects were asked to consume the meal at a steady pace,

142 with half of the meal consumed in the first 10 min, and the remainder of the meal consumed
143 in the 20 next min. This pacing should have ultimately reduced any variability in gastric
144 emptying due to variation in rates of intake. No other food was permitted over the following 8
145 h, but subjects were permitted to finish the remainder of the 330 mL of water they had not
146 drunk during the meal. A fasting baseline blood sample was drawn before administration of
147 the meal as well as at 2, 3, 4, 5, 6 and 8 h after meal consumption. Blood was taken up into
148 evacuated tubes containing K-EDTA. The tubes were immediately placed into an ice-water
149 bath and covered with aluminum foil to avoid light exposure. Plasma was isolated by
150 centrifugation (10 min at 4 °C and 878 g) within 2 h following collection.

151

152 *CM preparation*

153 Plasma (6 mL) was overlaid with 0.9% NaCl solution (4.5 mL) and centrifuged for 28
154 min at 130,000 x g at 10 °C using a SW41Ti rotor (Beckman Coulter, Villepinte, France) in a
155 Thermo Sorvall WX100 ultracentrifuge (Thermo Scientific, Saint Herblain, France). The
156 upper phase, containing mainly CM and large CM remnants [41, 42], was collected.
157 Immediately after recovery, CM were stored at -80 °C prior to LYC analysis.

158

159 *CM LYC extraction and analysis*

160 Total CM LYC, a mixture of all-trans and LYC cis isomers [42, 43], was extracted and
161 analyzed as follows. Briefly, up to 2 mL of CM was deproteinated by adding one volume of
162 ethanol which also contained apo-8'-carotenal as an internal standard. After adding two
163 volumes of hexane, the mixture was vortexed for 10 min and centrifuged at 500 x g for 10
164 min at 4 °C. The upper phase (containing LYC) was collected and the sample was extracted a
165 second time with hexane following the same procedure. The hexane phases were pooled and
166 evaporated completely under nitrogen gas. The dried extract was then redissolved in 200 µL

167 of a dichloromethane/methanol mixture (65:35, v/v). All extractions were performed at room
168 temperature under yellow light to minimize light-induced damage. A volume of 90 μ L was
169 injected for HPLC analysis. Separation was achieved using a 10 \times 4.0 mm, 2 μ m Modulo-
170 Cart QS guard column (Interchim, Montluçon, France) followed by a 250 \times 4.6 mm internal
171 diameter, 5 μ m particle size, YMC C30 column (Interchim) held at 35 °C. The mobile phase
172 was composed of HPLC grade methanol (A), methyl *tert*-butyl ether (B) and water (C). A
173 linear gradient of 96% A, 2% B, 2% C at t = 0 to 18% A, 80% B, 2% C at t = 27 min at a flow
174 rate of 1 mL/min was used. The HPLC system consisted of a pump (Waters 2690) connected
175 in-line with a photodiode-array detector (Waters 2996) (Waters, Saint Quentin en Yvelines,
176 France). LYC was identified via UV-Vis spectra and retention time coincident with authentic
177 standard (generous gift of DSM LTD, Basel, Switzerland) and quantitated at 472 nm. The
178 consecutive peaks, which contained all-trans LYC and cis-LYC isomers, were integrated
179 together to obtain the sum of all LYC isomers. Integration was performed using Chromeleon
180 software (version 6.80, Dionex, Villebon sur Yvette, France), and quantitation was performed
181 by comparing sample peak area with all-trans LYC calibration curves. Values were corrected
182 by extraction efficiency based on the recovery of the internal standard.

183

184 *Calculations*

185 The trapezoidal approximation rule was used to calculate the postprandial plasma CM
186 LYC response, i.e. the area of the postprandial plasma CM LYC concentration over 8 hours
187 (AUC). Calculated AUCs were baseline-corrected using the fasting plasma CM LYC
188 concentration measured in each subject.

189

190 *Choice of candidate genes*

191 The candidate genes included those whose encoded proteins have been shown by *in*
192 *vitro* methods to be involved in cellular uptake of LYC, *i.e.* *SCARB1* (scavenger receptor class
193 B, member 1) [19] and *CD36* (cluster of differentiation 36) [20], genes that are suspected to
194 be involved, directly or indirectly, in enterocyte LYC metabolism, *e.g.* *L-FABP* (liver-fatty
195 acid binding protein) [24], *MTTP* (microsomal triglyceride transfer protein), and genes that
196 have been associated in genome-wide association studies [23, 36] or candidate gene
197 association studies [30, 33-35] with blood LYC concentration. This resulted in the selection
198 of 26 genes (**Supplementary table 1**), representing 2202 SNPs on the arrays. In addition, we
199 added 30 SNPs in 23 genes that we have recently found to be associated with the postprandial
200 CM triacylglycerol response in the same group of subjects [44]. Indeed, CMs are the main
201 blood carrier of newly absorbed LYC and we hypothesized that genetic variants that affect the
202 secretion/clearance of CMs in the postprandial period likely affect the postprandial blood
203 response in LYC. We also added 40 SNPs that have been associated, in previous publications,
204 with lipid metabolism (**Supplementary table 2**). After genotyping of the subjects (see
205 above), SNPs whose genotype call rate was <95%, or SNPs presenting a significant departure
206 from Hardy-Weinberg equilibrium ($P < 0.05$ following the Chi-squared test), were excluded
207 from all subsequent analysis (387 SNPs excluded; 1885 SNPs left for the PLS regression
208 analysis).

209

210 *–Multivariate analysis with partial least squares regression*

211 In order to identify SNPs associated with the variability in the postprandial plasma
212 CM LYC response, we employed partial least squares regression (PLS). PLS is a multivariate
213 statistical tool often used for chemometric [45] and spectrometric modeling, and which has
214 recently been applied to SNP-based predictions by our group [44, 46] and others [47, 48]. Due
215 to the large number of SNPs compared with the low number of subjects and multicollinearity

216 between SNPs, PLS regression is used to identify the best combination of variables (i.e.
217 SNPs) that are predictive of another variable, in this case the postprandial CM LYC response.
218 Since the inheritance model could not be known for the 1885 SNPs entered in the PLS
219 regression analysis, a general genetic model was assumed, *i.e.* the three genotypes of each
220 SNP were treated as separate categories with no assumption made about the effect conferred
221 by homozygous or heterozygous alleles on the phenotype. Different PLS regression models
222 were built using increasing variable in the projection (VIP) threshold values. The model
223 maximizing the explained (R^2) and the predicted variance (Q^2), and validated following cross-
224 validation ANOVA, was selected. Additional validation procedures of the PLS regression
225 models [37, 44, 49] were also performed and are described in **Supplementary Methods**.
226 Simca-P12 software (Umetrics, Umeå, Sweden) was used for all multivariate data analyses
227 and modeling.

228

229 *Univariate analysis*

230 In a second approach, we performed univariate analyses to compare the postprandial
231 plasma CM LYC response between subgroups of subjects who bore different genotypes for
232 the SNPs selected in the PLS regression model. Differences obtained in the different genotype
233 subgroups were analyzed using Student's t-test with the Benjamini-Hochberg correction,
234 using QVALUE software [50]. For all tests, an adjusted P -value <0.05 was considered
235 significant.

236

237 **Results**

238 *Interindividual variability in postprandial plasma CM LYC responses to the test meal*

239 **Figure 1** shows the postprandial CM LYC response after consumption of the test meal
240 containing tomato puree. The CV of the postprandial plasma CM LYC response, called “LYC
241 response” hereafter in the manuscript, was 70%, as illustrated in **Figure 2**.

242

243 *Correlation between the LYC response to the test meal and the fasting plasma LYC*
244 *concentration*

245 In order to assess whether the fasting plasma LYC concentration, previously found to
246 be a good marker of LYC status, was related to LYC bioavailability, we calculated the
247 correlation between the LYC response of the subjects and their fasting plasma LYC
248 concentration. Results showed that there was a positive relationship between these two
249 variables (Pearson’s $r=0.37$, $P<0.05$).

250

251 *Genetic variants associated with the LYC response*

252 As explained in the subjects and methods section, we used PLS regression to examine
253 whether the 1885 candidate SNPs (used as qualitative X variables) could explain a significant
254 part of the variability in the LYC response of the subjects. As shown in **Table 2**, the model
255 including all SNPs explained the group variance with good accuracy ($R^2=0.95$) but was not
256 predictive of this variance ($Q^2=-0.1$). Therefore, to improve the model and find an association
257 of SNPs more predictive of the LYC response, we filtered out SNPs that made no important
258 contribution, *i.e.* those who displayed the lowest variable importance in the projection (VIP)
259 values. After applying several thresholds of VIP value (**Table 2**), we found that the best model
260 obtained included 44 SNPs (28 not in linkage disequilibrium, plus 16 in linkage
261 disequilibrium, shown in **Supplementary table 3**). The 28 SNPs were located in or near 16

262 genes (**Table 3**) and explained 72 % of the group variance, with a prediction index Q^2 of 56 %
263 (**Table 2**). The robustness and the stability of the model were validated by three additional
264 methods detailed in the **Supplementary Methods** section.

265 Using univariate statistics, the association of the 28 SNPs with the LYC response was
266 further evaluated by comparing, for each SNP, the LYC response of subjects who bore
267 different genotypes (**Table 3**). For 11 of the 28 SNPs, subjects who bore different genotypes
268 exhibited a significantly different LYC response (q-value<0.05).

269

270 *Genetic score to calculate the LYC response of a genotyped subject*

271 Knowing a subject's genotype at the 28 aforementioned loci, it is possible to calculate
272 his ability to respond to LYC, according to the following equation:

$$\widehat{RP} = a + \sum_1^{28} r_i * genotype.(SNP_i)$$

273 With RP as the responder phenotype (i.e. the LYC response), a as a constant, r_i as the
274 regression coefficient of the i^{th} SNP included in the PLS regression model, and
275 $genotype.(SNP_i)$ as a Boolean variable indicating the subject's genotype at the i^{th} SNP. A list
276 of the regression coefficients calculated by the SIMCA software can be found in
277 **Supplementary table 5**.

278

279 **Discussion**

280 The first key observation of this study was the high interindividual variability observed
281 in the LYC response to the test meal containing tomato puree. The 70% CV is in agreement
282 with previous reports [18, 26-30]. Until very recently, the reason for this high interindividual
283 variability was not known. Only one study has attempted to link this variability with SNPs in
284 two key genes of carotenoid metabolism, i.e. *BCO1* and *BCO2*, by observing plasma LYC
285 response following a 3 week supplementation with tomato or watermelon juice [30]. This data
286 suggested that subjects with the AA/CT genotype combination at rs12934922 and rs7501331
287 in *BCO1* were more responsive to LYC than those without this genotype combination.
288 However, numerous SNPs in genes related to other aspects of lycopene absorption and
289 excretion likely impact LYC response. Indeed, we have recently shown that 73% of the
290 interindividual variability in the bioavailability of the carotenoid lutein can be explained by a
291 combination of 29 SNPs in genes involved in lutein and lipid metabolism [37]. Moreover,
292 LYC bioavailability, is likely affected by multiple parameters besides LYC absorption, e.g.
293 catabolism, tissue uptake, oxidation, etc. Therefore, we assessed the involvement of SNPs in
294 all genes suspected to modulate this phenotype.

295 The result of the multivariate analysis, whose validity was verified by several tests,
296 demonstrated that a significant part (72%) of the interindividual variability in the LYC
297 response could be explained by 28 SNPs in or near 16 genes. Seven out of these 16 genes, i.e.
298 *ABCA1*, *LPL*, *INSIG2*, *SLC27A6*, *LIPC*, *CD36* and *APOB*, have recently been found to be
299 involved in the postprandial CM triacylglycerol response in the same group of subjects [44].
300 This was not surprising as most newly absorbed LYC is carried from the intestine to
301 peripheral organs via CM, which are mainly composed of triacylglycerols. Since this study
302 focuses on LYC bioavailability, we will only discuss the potential role of the 4 genes that
303 were not associated with the CM triacylglycerol response and that had SNPs which displayed

304 a significant corrected *P*-value (**Table 3**). These genes were *ELOVL2* (ELOVL fatty acid
305 elongase 2), *MTTP* (microsomal triglyceride transfer protein), *ABCB1* (ATP-binding cassette,
306 sub-family B, member 1) and *SOD2* (superoxide dismutase 2, mitochondrial). First, it is
307 striking to observe that 3 of the top 4 SNPs identified in the PLS model were related to
308 *ELOVL2*. This result suggests that this gene plays a key role in the LYC response. *ELOVL2* is
309 known to catalyze the elongation of EPA (eicosapentaenoic acid) to DPA (docosapentaenoic
310 acid) and DPA to DHA (docosahexaenoic acid). LYC is not considered to be a substrate for
311 this enzyme, thus, this association is intriguing. Nevertheless, the fact that rs3798709 and
312 rs9468304 in *ELOVL2* were also associated with both the lutein [37] and β -carotene response
313 (data not shown submitted to publication) strongly supports its involvement in carotenoid
314 metabolism. Three SNPs in *MTTP* were also found in the selected PLS model. *MTTP* encodes
315 for the microsomal triglyceride transfer protein, which is involved in the packaging of
316 triacylglycerols within the CM. Its association with the LYC response is therefore not
317 surprising, as LYC is incorporated into CM in the enterocyte. *ABCB1* encodes for the P-
318 glycoprotein, an ATP-dependent drug efflux pump for xenobiotic compounds with broad
319 substrate specificity. To the best of our knowledge, there is currently no study on the
320 involvement of this protein in LYC transport, but the present association suggests that this
321 protein may participate in LYC absorption, possibly by effluxing a fraction of uptaken LYC
322 back to the intestinal lumen. The low absorption efficiency of LYC, about 30% at the dose of
323 10 mg [18], supports this hypothesis, but further studies are required to determine ABCA1
324 involvement. Our results also suggest that *SOD2*, responsible for converting superoxide by-
325 products to hydrogen peroxide and diatomic oxygen, is involved in LYC status, possibly
326 through impacts on LYC metabolism. We hypothesize that when this enzyme is not efficient,
327 superoxide by-products are quenched by LYC, leading to its degradation.

328 Surprisingly, some genes assumed to be involved in intestinal LYC bioavailability and
329 metabolism were absent from the selected PLS model. These genes are *SCARB1* and *CD36*,
330 who code for proteins involved in the cellular uptake of LYC [19] [20], and *BCO2* [10] and
331 *BCO1* [22], which have been shown *in vitro* to cleave LYC. The fact that *SCARB1* was not
332 associated with LYC bioavailability was surprising as this scavenger receptor has been
333 involved in cellular uptake of LYC by intestinal cells [19, 51] and a recent study showed that
334 a SNP in this gene was associated with blood LYC concentration [36]. The results with *BCO1*
335 are in contradiction with the work by Wang et al., cited earlier [30] but match the findings for
336 *BCO2*. We have three main hypotheses to explain these lacks of association. The first
337 hypothesis is that SNPs genotyped in these genes do not result in a functionally different
338 phenotype with regards to LYC bioavailability. The second hypothesis is that the association
339 of SNPs in these genes is weaker than that of the SNPs in the selected model. In other words,
340 these SNPs may play a minor role in LYC bioavailability. This is not in disagreement with the
341 fact that they can play a significant role in fasting blood LYC concentrations. For example,
342 SR-BI is expressed in several tissues which could explain that genetic variants in this gene
343 play a key role in, for example, blood LYC uptake by other tissues while they do not play a
344 key role in LYC uptake by the intestine.. Finally, the third hypothesis is that some SNPs in
345 these genes were not entered in the PLS regression analysis because either they were not
346 expressed on the BeadChips, or they were excluded from the analysis (for not following the
347 Hardy-Weinberg equilibrium or because their genetic call rate was <95%). While we
348 acknowledge these limitations of the study, the primary results remain, i.e. a significant
349 fraction of the variability in LYC bioavailability is associated with a combination in genetic
350 variants identified in our model.

351 After determining that LYC bioavailability is, at least partly, modulated by a
352 combination of SNPs, the obvious question that arose was : “do these SNPs impacting LYC

353 bioavailability affect long-term blood LYC status?” To answer this question, we assessed
354 whether there was a relationship between the LYC response of the volunteers to the tomato
355 puree test meal and their fasting plasma LYC concentrations. The result of this investigation,
356 which showed a significant relationship between the two parameters, suggests that the ability
357 to respond to dietary LYC is implicated in long term blood, and likely tissue, LYC
358 concentrations. Nevertheless, the relatively low correlation coefficient, i.e. 0.37, supports the
359 assumption that other factors, e.g. LYC catabolism, oxidative stress..., are involved in long
360 term blood LYC concentrations.

361

362 To conclude, the results of this study demonstrate for the first time that interindividual
363 variability in LYC bioavailability is, at least partly, genetically controlled. They also suggest
364 that *ELOVL2* is implicated in LYC, and more generally, carotenoid metabolism. Furthermore,
365 the ability to respond to dietary LYC is a determinant of circulating fasting LYC
366 concentrations. These associations will need to be validated in other populations, which will
367 also allow for the identification of additional SNPs not included in our candidates based on
368 the current knowledge of LYC metabolism, which is still quite limited. The ultimate objective
369 of these studies will be to provide nutritionists with an accurate and validated genetic tool to
370 predict one’s individual LYC bioavailability for optimal LYC intake.

Tables

Table 1. Characteristics of the subjects included in the statistical analysis of the results.

<i>Parameter</i>	<i>Mean¹ ± SEM</i>
Age (years)	32.6 ± 2.1
Weight (kg)	72.6 ± 1.3
BMI (kg/m ²)	22.8 ± 0.3
Glucose (mM) ²	4.7 ± 0.1
Triacylglycerol (g/L) ²	0.7 ± 0.1
Total cholesterol (g/L) ²	1.7 ± 0.1
Hemoglobin (g/dL) ²	15.0 ± 0.1
LYC (μM) ^{2,3}	2.3 ± 0.3

¹ n=33.

² Fasting plasma variables.

LYC, lycopene.

Table 2. Performances of different partial least squares regression models to explain the postprandial chylomicron lycopene response. ¹

<i>VIP threshold</i>	<i>R</i> ²	<i>Q</i> ²	<i>SNPs no.</i>	<i>P (CV-ANOVA)</i>
No selection	0.94	-0.10	1885	1
>0.5	0.87	-0.10	604	1
>1.0	0.70	0.01	183	0.808
>1.5	0.73	0.57	61	0.109
>1.55	0.72	0.55	58	0.104
>1.6	0.71	0.52	56	0.104
>1.65	0.71	0.55	50	0.072
>1.7	0.72	0.55	45	0.041
>1.75	0.72	0.56	44	0.037
>1.8	0.70	0.45	32	0.047
>1.85	0.66	0.37	25	0.025
>1.9	0.63	0.36	23	0.037
>1.95	0.60	0.34	20	0.040
>2.0	0.62	0.39	16	0.036
>2.1	0.62	0.39	15	0.029

¹ Different PLS regression models were built using increasing VIP threshold values. The model maximizing the explained (R^2) and the predicted variance (Q^2), and validated following cross-validation ANOVA plus three other validation methods (described in **Supplementary Material**), was selected. This is the model with $VIP > 1.75$, shown in the table.

CV-ANOVA, cross-validation ANOVA; Q^2 , predicted variance; R^2 , explained variance; SNP, single nucleotide polymorphism; VIP, variable importance in the projection.

Table 3. Genes and single nucleotide polymorphisms associated with the postprandial chylomicron lycopene response. ¹

<i>Gene and SNP rs no.</i> ²	<i>VIP value</i>	<i>SNP minor allele frequency</i>	<i>Adjusted P-value</i> ³
<i>ELOVL2</i> -rs3798709	3.38	0.252	0.030
<i>ELOVL2</i> -rs9468304	3.33	0.302	0.030
<i>LPL</i> -rs7005359	3.19	0.298	0.030
<i>ELOVL2</i> -rs911196	3.07	0.252	0.030
<i>ABCA1</i> -rs4149316	2.81	0.122	0.023
<i>MTTP</i> -rs17029173	2.73	0.135	0.030
<i>ABCB1</i> -rs10248420	2.67	0.347	0.030
<i>PKD1L2</i> -rs935933	2.57	0.201	0.053
<i>MTTP</i> -rs1032355	2.56	0.251	0.067
<i>ABCG2</i> -rs1871744	2.55	0.173	0.061
<i>ABCA1</i> -rs2791952	2.50	0.140	0.030
<i>LPL</i> -rs7841189	2.44	0.148	0.063
<i>INSIG2</i> -rs17006621	2.27	0.172	0.077
<i>ABCA1</i> -rs3887137	2.15	0.123	0.076
<i>MTTP</i> -rs745075	2.03	0.061	0.030
<i>NPC1L1</i> -rs17725246	1.97	0.254	0.106
<i>LPL</i> -rs17482753	1.95	0.088	0.101
<i>SOD2</i> -rs9365046	1.93	0.169	0.039
<i>SLC27A6</i> -rs10053477	1.91	0.209	0.090
<i>ABCA1</i> -rs1331924	1.88	0.245	0.091
<i>ISX</i> -rs2056983	1.85	0.117	0.092

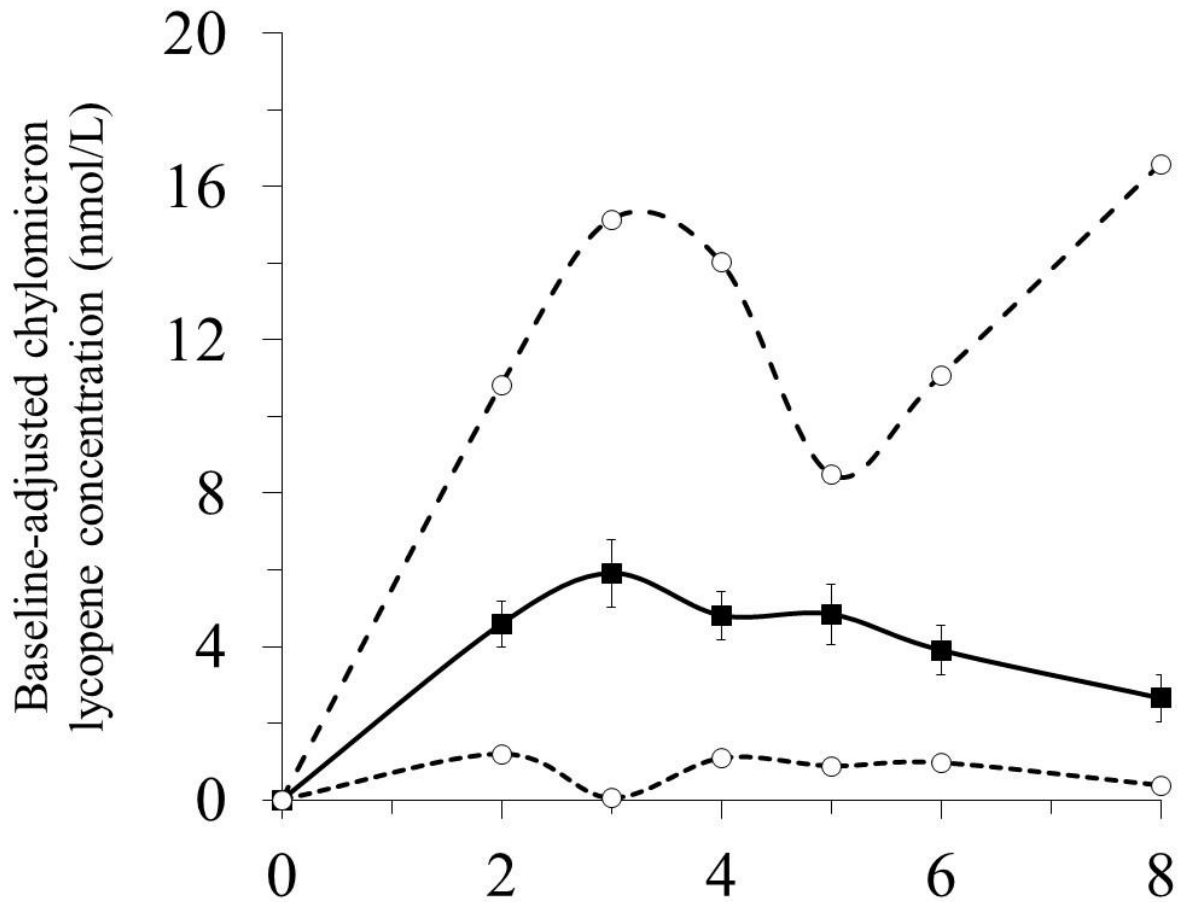
<i>LIPC</i> -rs12914035	1.85	0.096	0.146
<i>CD36</i> -rs4112274	1.85	0.224	0.030
<i>PNLIP</i> -rs11197742	1.84	0.087	0.086
<i>ABCA1</i> -rs4149299	1.84	0.082	0.119
<i>APOB</i> -rs1042031	1.76	0.128	0.157
<i>LIPC</i> -rs8035357	1.76	0.150	0.101
<i>ABCB1</i> -rs10280101	1.75	0.145	0.101

¹ SNPs present in the selected PLS regression model shown in **Table 2**.

² SNPs are ranked by decreasing VIP value. Note that 16 out of the 44 SNPs present in the selected model were in linkage disequilibrium. Since these SNPs provided redundant information, we randomly kept one of each SNP (those presented in this table) in the final selected PLS model. The SNPs in linkage disequilibrium with some of these SNPs are shown in **Supplemental table 2**.

³ Student's t-test with the Benjamini-Hochberg correction was carried out to determine differences between the postprandial CM LYC response according to genotype groups for each SNP.

SNP, single nucleotide polymorphism; rs, reference SNP; VIP, variable importance in the projection. A complete list of gene names and symbols can be found in **Supplementary table 1**.



Figures

Figure 1: Baseline-adjusted chylomicron lycopene (CM LYC) concentration over 8 h after consumption of the test meal containing tomato puree.

For each subject, postprandial CM LYC concentrations were baseline-adjusted by normalizing to the fasting CM LYC concentration. The bold curve shows mean \pm SEM of 33 subjects combined. The smaller dashed curve shows the concentration of CM LYC measured in the lowest responder. The larger dashed curve shows the concentration of CM LYC measured in the highest responder.

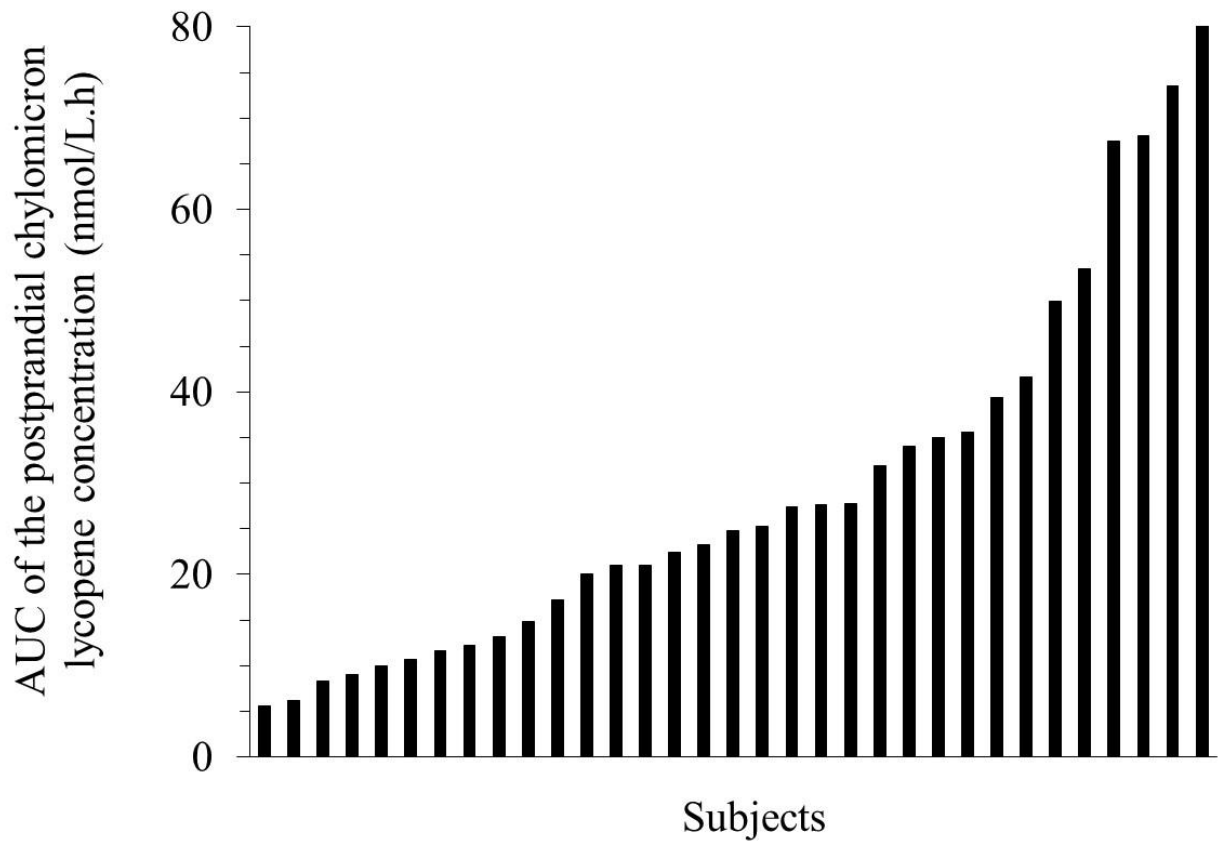


Figure 2: Individual AUCs of the postprandial CM LYC response after consumption of the test meal containing tomato puree.

Subjects were sorted by increasing postprandial CM LYC response (i.e., 0-8 h AUC).

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Authors' contributions to manuscript:

PB designed research; MN conducted the clinical research; RB analysed lycopene by HPLC; CD and PB analyzed data and CD performed statistical analysis; PB and CD wrote the paper; PB and CD had primary responsibility for final content.

References

- [1] Story, E. N.; Kopec, R. E.; Schwartz, S. J.; Harris, G. K. An update on the health effects of tomato lycopene. *Annu. Rev. Food Sci. Technol.* **1**:189-210; 2010.
- [2] Mordente, A.; Guantario, B.; Meucci, E.; Silvestrini, A.; Lombardi, E.; Martorana, G. E.; Giardina, B.; Bohm, V. Lycopene and cardiovascular diseases: an update. *Curr. Med. Chem.* **18**:1146-1163; 2011.
- [3] Bohm, V. Lycopene and heart health. *Mol. Nutr. Food Res.* **56**:296-303; 2012.
- [4] Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. *J. Natl. Cancer Inst.* **91**:317-331; 1999.
- [5] Viuda-Martos, M.; Sanchez-Zapata, E.; Sayas-Barbera, E.; Sendra, E.; Perez-Alvarez, J. A.; Fernandez-Lopez, J. Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. *Crit. Rev. Food Sci. Nutr.* **54**:1032-1049; 2014.
- [6] Biddle, M. J.; Lennie, T. A.; Bricker, G. V.; Kopec, R. E.; Schwartz, S. J.; Moser, D. K. Lycopene Dietary Intervention: A Pilot Study in Patients With Heart Failure. *J. Cardiovasc. Nurs.*; 2014.
- [7] Kelkel, M.; Schumacher, M.; Dicato, M.; Diederich, M. Antioxidant and anti-proliferative properties of lycopene. *Free Radic. Res.* **45**:925-940; 2011.
- [8] Kopec, R. E.; Riedl, K. M.; Harrison, E. H.; Curley, R. W., Jr.; Hruszkewycz, D. P.; Clinton, S. K.; Schwartz, S. J. Identification and quantification of apo-lycopenals in fruits, vegetables, and human plasma. *J. Agric. Food Chem.* **58**:3290-3296; 2010.
- [9] Erdman, J. W., Jr.; Ford, N. A.; Lindshield, B. L. Are the health attributes of lycopene related to its antioxidant function? *Arch. Biochem. Biophys.* **483**:229-235; 2009.

- [10] Lindshield, B. L.; Canene-Adams, K.; Erdman, J. W., Jr. Lycopene metabolites bioactive? *Arch. Biochem. Biophys.* **458**:136-140; 2007.
- [11] Mein, J. R.; Lian, F.; Wang, X. D. Biological activity of lycopene metabolites: implications for cancer prevention. *Nutr. Rev.* **66**:667-683; 2008.
- [12] Gouranton, E.; Thabuis, C.; Riollet, C.; Malezet-Desmoulins, C.; El Yazidi, C.; Amiot, M. J.; Borel, P.; Landrier, J. F. Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue. *J. Nutr. Biochem.* **22**:642-648; 2011.
- [13] Aydemir, G.; Kasiri, Y.; Birta, E.; Beke, G.; Garcia, A. L.; Bartok, E. M.; Ruhl, R. Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-activating metabolites may be relevant for lycopene's anti-cancer potential. *Mol. Nutr. Food Res.* **57**:739-747; 2013.
- [14] Gouranton, E.; Aydemir, G.; Reynaud, E.; Marcotorchino, J.; Malezet, C.; Caris-Veyrat, C.; Blomhoff, R.; Landrier, J. F.; Ruhl, R. Apo-10'-lycopenoic acid impacts adipose tissue biology via the retinoic acid receptors. *Biochim. Biophys. Acta* **1811**:1105-1114; 2012.
- [15] Wang, X. D. Lycopene metabolism and its biological significance. *Am. J. Clin. Nutr.* **96**:1214S-1222S; 2012.
- [16] Yeum, K. J.; Russell, R. M. Carotenoid bioavailability and bioconversion. *Annu. Rev. Nutr.* **22**:483-504; 2002.
- [17] Borel, P. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin. Chem. Lab. Med.* **41**:979-994; 2003.
- [18] Diwadkar-Navsariwala, V.; Novotny, J. A.; Gustin, D. M.; Sosman, J. A.; Rodvold, K. A.; Crowell, J. A.; Stacewicz-Sapuntzakis, M.; Bowen, P. E. A physiological

- pharmacokinetic model describing the disposition of lycopene in healthy men. *J. Lipid Res.* **44**:1927-1939; 2003.
- [19] Moussa, M.; Landrier, J. F.; Reboul, E.; Ghiringhelli, O.; Comera, C.; Collet, X.; Frohlich, K.; Bohm, V.; Borel, P. Lycopene absorption in human intestinal cells and in mice involves scavenger receptor class B type I but not Niemann-Pick C1-like 1. *J. Nutr.* **138**:1432-1436; 2008.
- [20] Moussa, M.; Gouranton, E.; Gleize, B.; Yazidi, C. E.; Niot, I.; Besnard, P.; Borel, P.; Landrier, J. F. CD36 is involved in lycopene and lutein uptake by adipocytes and adipose tissue cultures. *Mol. Nutr. Food Res.* **55**:578-584; 2011.
- [21] dela Sena, C.; Riedl, K. M.; Narayanasamy, S.; Curley, R. W., Jr.; Schwartz, S. J.; Harrison, E. H. The human enzyme that converts dietary provitamin A carotenoids to vitamin A is a dioxygenase. *J. Biol. Chem.* **289**:13661-13666; 2014.
- [22] dela Sena, C.; Narayanasamy, S.; Riedl, K. M.; Curley, R. W., Jr.; Schwartz, S. J.; Harrison, E. H. Substrate specificity of purified recombinant human beta-carotene 15,15'-oxygenase (BCO1). *J. Biol. Chem.* **288**:37094-37103; 2013.
- [23] Ferrucci, L.; Perry, J. R.; Matteini, A.; Perola, M.; Tanaka, T.; Silander, K.; Rice, N.; Melzer, D.; Murray, A.; Cluett, C.; Fried, L. P.; Albanes, D.; Corsi, A. M.; Cherubini, A.; Guralnik, J.; Bandinelli, S.; Singleton, A.; Virtamo, J.; Walston, J.; Semba, R. D.; Frayling, T. M. Common Variation in the beta-Carotene 15,15'-Monooxygenase 1 Gene Affects Circulating Levels of Carotenoids: A Genome-Wide Association Study. *Am. J. Hum. Genet.* **84**:123-133; 2009.
- [24] Reboul, E.; Borel, P. Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. *Prog. Lipid Res.* **50**:388-402; 2011.

- [25] Dallinga-Thie, G. M.; Franssen, R.; Mooij, H. L.; Visser, M. E.; Hassing, H. C.; Peelman, F.; Kastelein, J. J.; Peterfy, M.; Nieuwdorp, M. The metabolism of triglyceride-rich lipoproteins revisited: new players, new insight. *Atherosclerosis* **211**:1-8; 2013.
- [26] Stahl, W.; Sies, H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *J. Nutr.* **121**:2161-2166; 1992.
- [27] Bowen, P.; Garg, V.; Stacewiczapuntzakis, M.; Yelton, L.; Schreiner, R. S. Variability of Serum Carotenoids in Response to Controlled Diets Containing Six Servings of Fruits and Vegetables per day. *Ann. NY Acad. Sci.* **691**:241-243; 1993.
- [28] Johnson, E. J.; Qin, J.; Krinsky, N. I.; Russell, R. M. Ingestion by men of a combined dose of beta-carotene and lycopene does not affect the absorption of beta-carotene but improves that of lycopene. *J. Nutr.* **127**:1833-1837; 1997.
- [29] O'Neill, M. E.; Thurnham, D. I. Intestinal absorption of β -carotene, lycopene and lutein in men and women following a standard meal: response curves in the triacylglycerol-rich lipoprotein fraction. *Br. J. Nutr.* **79**:149-159; 1998.
- [30] Wang, T. T.; Edwards, A. J.; Clevidence, B. A. Strong and weak plasma response to dietary carotenoids identified by cluster analysis and linked to beta-carotene 15,15'-monooxygenase 1 single nucleotide polymorphisms. *J. Nutr. Biochem.* **24**:1538-1546; 2013.
- [31] Borel, P. Genetic variations involved in interindividual variability in carotenoid status. *Mol. Nutr. Food Res.* **56**:228-240; 2012.
- [32] Moran, N. E.; Erdman, J. W., Jr.; Clinton, S. K. Complex interactions between dietary and genetic factors impact lycopene metabolism and distribution. *Arch. Biochem. Biophys.* **539**:171-180; 2013.

- [33] Ortega, H.; Castilla, P.; Gomez-Coronado, D.; Garces, C.; Benavente, M.; Rodriguez-Artalejo, F.; de Oya, M.; Lasuncion, M. A. Influence of apolipoprotein E genotype on fat-soluble plasma antioxidants in Spanish children. *Am. J. Clin. Nutr.* **81**:624-632; 2005.
- [34] Borel, P.; Moussa, M.; Reboul, E.; Lyan, B.; Defoort, C.; Vincent-Baudry, S.; Maillot, M.; Gastaldi, M.; Darmon, M.; Portugal, H.; Planells, R.; Lairon, D. Human plasma levels of vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid metabolism. *J. Nutr.* **137**:2653-2659; 2007.
- [35] Borel, P.; Moussa, M.; Reboul, E.; Lyan, B.; Defoort, C.; Vincent-Baudry, S.; Maillot, M.; Gastaldi, M.; Darmon, M.; Portugal, H.; Lairon, D.; Planells, R. Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, cholesteryl ester transfer protein, hepatic lipase, intestinal fatty acid binding protein and microsomal triacylglycerol transfer protein. *Br. J. Nutr.* **101**:680-687; 2009.
- [36] Zubair, N.; Kooperberg, C.; Liu, J.; Di, C.; Peters, U.; Neuhausser, M. L. Genetic variation predicts serum lycopene concentrations in a multiethnic population of postmenopausal women. *J. Nutr.* **145**:187-192; 2015.
- [37] Borel, P.; Desmarchelier, C.; Nowicki, M.; Bott, R.; Morange, S.; Lesavre, N. Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relation with fasting plasma lutein concentration. *Am. J. Clin. Nutr.* **100**:168-175; 2014.
- [38] Porrini, M.; Riso, P. What are typical lycopene intakes? *J. Nutr.* **135**:2042S-2045S; 2005.
- [39] Hansen, T. V.; Simonsen, M. K.; Nielsen, F. C.; Hundrup, Y. A. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison

- of the response rate and quality of genomic DNA. *Cancer Epidemiol. Biomarkers Prev.* **16**:2072-2076; 2007.
- [40] Lecompte, S.; Szabo de Edelenyi, F.; Goumidi, L.; Maiani, G.; Moschonis, G.; Widhalm, K.; Molnár, D.; Kafatos, A.; Spinneker, A.; Breidenassel, C.; Dallongeville, J.; Meirhaeghe, A.; Borel, P. Polymorphisms in the CD36/FAT gene are associated with plasma vitamin E levels in humans. *Am. J. Clin. Nutr.* **93**:1-8; 2011.
- [41] Luchoomun, J.; Hussain, M. M. Assembly and secretion of chylomicrons by differentiated Caco-2 cells. Nascent triglycerides and preformed phospholipids are preferentially used for lipoprotein assembly. *J. Biol. Chem.* **274**:19565-19572; 1999.
- [42] Richelle, M.; Lambelet, P.; Rytz, A.; Tavazzi, I.; Mermoud, A. F.; Juhel, C.; Borel, P.; Bortlik, K. The proportion of lycopene isomers in human plasma is modulated by lycopene isomer profile in the meal but not by lycopene preparation. *Br. J. Nutr.* **107**:1482-1488; 2012.
- [43] Richelle, M.; Sanchez, B.; Tavazzi, I.; Lambelet, P.; Bortlik, K.; Williamson, G. Lycopene isomerisation takes place within enterocytes during absorption in human subjects. *Br. J. Nutr.* **103**:1800-1807; 2010.
- [44] Desmarchelier, C.; Martin, J. C.; Planells, R.; Gastaldi, M.; Nowicki, M.; Goncalves, A.; Valero, R.; Lairon, D.; Borel, P. The postprandial chylomicron triacylglycerol response to dietary fat in healthy male adults is significantly explained by a combination of single nucleotide polymorphisms in genes involved in triacylglycerol metabolism. *J. Clin. Endocrinol. Metab.* **99**:E484-488; 2014.
- [45] Eriksson, L.; Antti, H.; Gottfries, J.; Holmes, E.; Johansson, E.; Lindgren, F.; Long, I.; Lundstedt, T.; Trygg, J.; Wold, S. Using chemometrics for navigating in the large data sets of genomics, proteomics, and metabonomics (gpm). *Anal. Bioanal. Chem.* **380**:419-429; 2004.

- [46] Borel, P.; de Edelenyi, F. S.; Vincent-Baudry, S.; Malezet-Desmoulin, C.; Margotat, A.; Lyan, B.; Gorrard, J. M.; Meunier, N.; Drouault-Holowacz, S.; Bieuvelet, S. Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans. *Ann. Med.* **43**:47-59; 2010.
- [47] Le Cao, K. A.; Boitard, S.; Besse, P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinformatics* **12**:253; 2011.
- [48] Long, N.; Gianola, D.; Rosa, G. J.; Weigel, K. A. Dimension reduction and variable selection for genomic selection: application to predicting milk yield in Holsteins. *J. Anim. Breed Genet.* **128**:247-257; 2011.
- [49] Borel, P.; Desmarchelier, C.; Nowicki, M.; Bott, R.; Tourniaire, F. Can genetic variability in alpha-tocopherol bioavailability explain the heterogeneous response to alpha-tocopherol supplements? *Antioxid. Redox. Signal.*:Nov 12. [Epub ahead of print]; 2014.
- [50] Storey, J. D. A direct approach to false discovery rates. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **64**:479-498; 2002.
- [51] During, A.; Dawson, H. D.; Harrison, E. H. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. *J. Nutr.* **135**:2305-2312; 2005.