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Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relationship with fasting plasma lutein concentration.

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Running head: SNPs involved in lutein bioavailability

1 **Abbreviations:** ABCG2 (ATP-binding cassette, sub-family G, member 2); (ABCG5/G8)
 2 ATP Binding Cassette G5/G8; ABCA1 (ATP Binding Cassette sub-family member 1); AUC
 3 (area under the curve); BCMO1 (β,β -carotene-15,15'-monooxygenase); BCO2 (β,β -carotene-
 4 9,10'-oxygenase); BCRP (breast cancer resistance protein); CD36 (cluster determinant 36);
 5 CM (chylomicron); ELOVL2 (ELOVL fatty acid elongase 2); HPLC (high performance
 6 liquid chromatography); I-FABP (intestinal-fatty acid binding protein); ISX (Intestine
 7 Specific Homeobox); L-FABP (liver-fatty acid binding protein); LUT (lutein); MTTP
 8 (microsomal triglyceride transfer protein); NPC1L1 (Niemann-Pick disease, type C1, gene-
 9 like 1); PLS (partial least squares); SCARB1 (Scavenger receptor class B, member 1); SNPs
 10 (single nucleotide polymorphisms); SR-BI (scavenger receptor class B type I); VIP (variable
 11 importance in the projection).

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

12 **Abstract**

13 **Background:** Lutein (LUT) accumulates in the macula and in the brain where it is assumed
14 to play physiological roles. Its bioavailability is assumed to display a high interindividual
15 variability that is hypothesized to be due, at least partly, to genetic polymorphisms.

16 **Objectives:** i) to characterize interindividual variability in LUT bioavailability in humans, ii)
17 to assess the relationship between this variability and fasting blood LUT concentration, iii) to
18 identify single nucleotide polymorphisms (SNPs) involved in this phenomenon.

19 **Design:** In a randomized, 2-way crossover study, 39 healthy males consumed a meal
20 containing a LUT supplement, or the same meal where LUT was provided through tomato
21 puree. LUT concentration was measured in plasma chylomicrons (CM) isolated at regular
22 time intervals over 8 h postprandially. Multivariate statistical analyses were used to identify a
23 combination of SNPs associated with the postprandial CM LUT response (0 to 8 h area under
24 the curve). 1785 SNPs in 51 candidate genes were selected.

25 **Results:** The postprandial CM LUT responses to the meals were very variable (CV of 75%
26 and 137% for the LUT supplement and the tomato-sourced LUT meal, respectively). The
27 postprandial CM LUT responses measured after the two meals were positively correlated
28 ($r=0.68$, $P<0.0001$) and were positively correlated to the fasting plasma LUT concentration
29 ($r=0.51$, $P<0.005$ for the LUT supplement-containing meal). A significant ($P=1.9 \times 10^{-4}$) and
30 validated partial least squares regression model, which included 29 SNPs in 15 genes, could
31 explain most of the variance in the postprandial CM LUT response.

32 **Conclusions:** The ability to respond to LUT appears to be at least in part genetically
33 determined. It is explained in large part by a combination of SNPs in 15 genes related to both
34 LUT and CM metabolism. Finally, our results suggest that the ability to respond to LUT and
35 the blood LUT status are related.

36

37 **Introduction**

38 Lutein (LUT) is found at high concentrations in the human macula lutea (1-6). Its involvement
39 in the prevention of age-related macular degeneration has been suggested (7-13). LUT has
40 recently been found in the human brain where it has been suggested to have a beneficial role
41 on cognitive function (14, 15).

42 Clinical studies that have provided dietary LUT have reported high interindividual
43 variability in blood and tissue LUT levels in response to dietary LUT (16). Both dietary
44 factors (17) and genetic variations between individuals (18) have been proposed to explain
45 this phenomenon. In support of the latter factor, recent studies have shown that blood and
46 tissue levels of LUT are associated with genetic polymorphisms (16, 19, 20).

47 LUT metabolism starts in the gastrointestinal lumen where digestive enzymes can
48 modulate its bioaccessibility by allowing the release of this hydrophobic compound from its
49 food matrix to mixed micelles (17, 21). Cell culture studies have shown that at least two
50 apical membrane transport proteins, SR-BI (scavenger receptor class B type I) (22, 23), which
51 is encoded by *SCARB1*, and NPC1L1 (Niemann-Pick disease, type C1, gene-like 1) (24),
52 participate in the uptake of micellarized LUT. Candidate gene association studies have also
53 suggested that other enterocyte membrane lipid transporters, *i.e.* cluster determinant 36
54 (CD36) (19), ATP Binding Cassette G5/G8 (ABCG5/G8) (19, 20) and ATP Binding Cassette
55 sub-family member 1 (ABCA1) (16, 20), could participate in LUT absorption.

56 After its uptake at the apical side of the enterocyte, LUT is transported within the cell
57 to the site where it is incorporated into chylomicrons (CM). The precise mechanisms of this
58 process are not known, although proteins involved in intracellular transport of lipids might be
59 involved (25).

60 The enterocyte contains two enzymes that can be involved in carotenoid metabolism
61 within the enterocyte: BCMO1 (β,β -carotene-15,15'-monooxygenase), which has been

62 associated with blood LUT status (16, 19, 26-28), and BCO2 (β,β -carotene-9,10'-oxygenase),
63 whose involvement in LUT metabolism has recently been demonstrated (29). The fraction of
64 carotenoid that is not metabolized by these enzymes is incorporated into CM, which are
65 secreted into the lymph and then enter the bloodstream. CM are then hydrolyzed to CM
66 remnants that are mainly taken up by liver cells (30).

67 While it is known that there is high interindividual variability in LUT bioavailability
68 no study has attempted to comprehensively characterize this phenomenon. Furthermore, the
69 consequences of this variability with regard to chronic blood LUT status are not known. Thus,
70 the aims were i) to better characterize interindividual variability of LUT bioavailability in
71 healthy subjects, ii) to assess whether this variability in LUT bioavailability can affect fasting
72 concentrations of LUT, and iii) to identify the interplay between the multiple genetic variants
73 that are involved in this phenomenon.

74

75 **Subjects and Methods**

76 *Subject number and characteristics*

77 Forty healthy, nonobese, nonsmoker male subjects were recruited for the study. This
78 group of subjects had allowed to identify a combination of single nucleotide polymorphisms
79 (SNPs) that significantly explains the postprandial CM triacylglycerol response to dietary fat
80 (31).The subjects presented normal energy consumption, i.e. \approx 2500 kcal/d. They either drank
81 no alcohol or $<2\%$ alcohol as total energy. Subjects had no history of chronic disease,
82 hyperlipidemia, hyperglycemia, and were not taking any medication known to affect LUT or
83 lipid metabolism during the month prior to the study or during the study period. Because of
84 the relatively large volume of blood that was drawn during the study, subjects were required
85 to have a blood hemoglobin concentration >1.3 g/L as inclusion criteria. The study was
86 approved by the regional committee on human experimentation (N°2008-A01354-51, Comité
87 de Protection des Personnes Sud Méditerranée I, France). The procedures followed were in
88 accordance with the Helsinki Declaration of 1975 as revised in 1983. The objectives and
89 requirements of the study were fully explained to all participants before beginning the study,
90 and informed written consent was obtained from each subject. One subject left the study for
91 personal reason, before he participated in the first postprandial experiment, leaving 39
92 subjects whose baseline characteristics are reported in **Table 1**.

93

94 *DNA preparation and genotyping methods*

95 An average of 25 μ g of DNA was isolated from a saliva sample from each subject
96 using the Oragene kit (DNA Genotek Inc., Kanata, ON, Canada) as described in detail
97 previously (32). DNA concentration and purity were checked by spectrophotometry at 260 nm
98 and 280 nm (Nanodrop ND1000, Thermo Scientific, Villebon sur Yvette, France). All
99 genotyping procedures were carried out by Integragen (Evry, France). Concerning the whole

100 genome genotyping the procedure was as follows: 200 ng of DNA was hybridized overnight
101 to HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA), allowing the analysis of
102 approximately 7.33×10^5 SNPs per DNA sample. Unhybridized and non-specifically
103 hybridized DNA was washed out. The BeadChips were then stained and scanned on an
104 Illumina iScan. Detailed methods are provided in the Infinium HD Assay Ultra Protocol
105 Guide from Illumina. Concerning the 43 other SNPs (see below), they were genotyped as
106 previously described (33).

107

108 *Postprandial experiments*

109 For the clinical research study, we used a randomized, 2-way crossover design with a
110 washout period of 3 weeks minimum between each meal. Subjects were asked to refrain from
111 consuming LUT-rich foods (an exclusion list was provided by a dietitian) for 48 h before each
112 postprandial clinic visit. In addition, the day prior to the postprandial clinic visit, subjects
113 were asked to eat dinner between 7 and 8 p.m., without any alcohol intake, and afterward to
114 abstain from consuming any food or beverage other than water until their clinic visit. After
115 the overnight fast, subjects arrived at the local Center for Clinical Investigation (la
116 Conception Hospital, Marseille, France) and consumed either a meal that provided LUT as a
117 supplement or a meal that provided LUT in a food matrix, i.e. tomato puree. Both meals
118 consisted of semolina (70 g) cooked in 200 mL of hot water, white bread (40 g), egg whites
119 (60 g), peanut oil (50 g), and mineral water (330 mL). The LUT supplement was provided in
120 three pills, each containing 5 mg of free LUT (Visiobane Protect, Pileje, France). The source
121 of free LUT in the pills was FloraGLO (Kemin industry, Inc., Des Moines, IA, USA). The
122 pills also contained *Porphyra*, B vitamins, vitamins C and E, fish oil, bee wax, and gelatin.
123 The tomato puree (100 g/meal), purchased from a local supermarket, provided 0.1 mg free
124 LUT as measured by High Pressure Liquid Chromatography (HPLC). Tomato puree was

125 chosen as a source of food LUT because it has been shown that a postprandial CM LUT
126 response can efficiently be measured after tomato puree consumption (34) and because we
127 used this food in a parallel study dedicated to lycopene bioavailability (data presented in a
128 future paper on tomato lycopene bioavailability). Thirty three out of the 39 subjects
129 participated in the study on tomato lycopene bioavailability and thus consumed the tomato
130 puree. Subjects were asked to consume the meals at a steady pace, with half of the meal
131 consumed in 10 min and the remainder of the meal consumed within 30 min, in order to
132 diminish variability due to different rates of intake and thus gastric emptying. No other food
133 was permitted over the following 8 h. The subjects were only allowed to finish the remaining
134 bottled water they had not drunk during the meal. A baseline blood sample was drawn before
135 administration of the meals, *i.e.* in the fasted state, as well as at 2, 3, 4, 5, 6 and 8 h after meal
136 consumption. Blood was taken up into evacuated tubes containing K-EDTA. The tubes were
137 immediately placed into an ice-water bath and covered with aluminum foil to avoid light
138 exposure. Plasma was isolated by centrifugation (10 min at 4 °C and 878 g) within 2 h
139 following collection.

140

141 *CM preparation*

142 Plasma (6 mL) was overlaid with 0.9% NaCl solution (4.5 mL) and centrifuged for 28
143 min at 130,000 g at 10 °C using a SW41Ti rotor (Beckman Coulter, Villepinte, France) in a
144 Thermo Sorvall WX100 ultracentrifuge (Thermo Scientific, Saint Herblain, France). The
145 upper phase, containing mainly CM and large CM remnants (35, 36), was collected.
146 Immediately after recovery, CM were stored at -80 °C prior to LUT analysis.

147

148 *CM LUT extraction and analysis*

149 CM LUT was extracted and analyzed as follows. Briefly, up to 2 mL of CM were
150 deproteinated by adding one volume of ethanol containing apo 8'-carotenal as an internal
151 standard. After adding two volumes of hexane, the mixture was vortexed for 10 min. and
152 centrifuged at 500 g for 10 min at 4 °C. The upper phase (containing LUT) was collected, and
153 the sample was extracted a second time with hexane following the same procedure. The
154 hexane phases were pooled and evaporated completely under nitrogen gas. The dried extract
155 was then redissolved in 200 µL of a dichloromethane/methanol mixture (65/35; v/v). All
156 extractions were performed at room temperature under yellow light to minimize light-induced
157 damage. A volume of 90 µL was used for HPLC analysis. Separation was achieved using a 10
158 × 4.0 mm, 2 µm Modulo-Cart QS guard column (Interchim, Montluçon, France) followed by
159 a 250 × 4.6 mm internal diameter, 5 µm particle size, YMC C30 column (Interchim) held at
160 35 °C. The mobile phase was composed of HPLC grade methanol (A), methyl *tert*-butyl
161 ether (B) and water (C) (Carlo Erba – SDS, Peypin, France). A linear gradient from 96% A,
162 2% B, 2% C at t = 0 to 18% A, 80% B, 2% C at t = 27 min at a flow rate of 1 mL/min was
163 used. The HPLC system consisted of a pump (Waters 2690) associated with a photodiode-
164 array detector (Waters 2996) (Waters, Saint Quentin en Yvelines, France). LUT was
165 identified via spectra and retention time coincident with authentic standard (generous gift of
166 DSM LTD, Basel, Switzerland) and quantitated at 450 nm. Peaks were integrated using
167 Chromeleon software (version 6.80, Dionex, Villebon sur Yvette, France), and quantitation
168 was performed by comparing sample peak area with LUT calibration curves and corrected by
169 extraction efficiency based on the recovery of internal standard.

170

171 *Calculations*

172 Trapezoidal rule was used to calculate the postprandial plasma CM LUT response, i.e.
173 the AUC of the postprandial plasma CM LUT concentration over 8 hours. Calculated AUCs
174 were incremental, i.e. baseline-corrected using the fasting plasma CM LUT concentration
175 measured in each subject.

176

177 *Choice of candidate genes*

178 The candidate genes included those whose encoded proteins have been shown by *in*
179 *vitro* methods to be involved in LUT uptake by the enterocyte, *i.e.* *SCARB1* (Scavenger
180 receptor class B, member 1) and *NPC1L1* (22, 24), genes that are suspected to be involved,
181 directly or indirectly, in enterocyte LUT metabolism, *e.g.* *L-FABP* (liver-fatty acid binding
182 protein), *I-FABP* (intestinal-fatty acid binding protein)(25), *MTTP* (microsomal triglyceride
183 transfer protein), and genes that have been associated in genome-wide association studies (26)
184 or candidate gene association studies (18, 28, 37) with blood LUT concentration. This
185 resulted in the selection of 28 genes (**Supplementary table 1**), representing 2091 SNPs on
186 the arrays. In addition, we added 30 SNPs in 23 genes that we have recently found to be
187 associated with the postprandial CM triacylglycerol response in the same group of subjects
188 (31). Indeed, CMs are the main blood carrier of newly absorbed LUT and we hypothesized
189 that genetic variants that affect the secretion/clearance of CMs in the postprandial period
190 likely affect the postprandial blood response in LUT. We also added 43 SNPs that have been
191 associated, in previous publications, with lipid metabolism (**Supplementary table 2**). After
192 genotyping of the subjects (see above), SNPs whose genotype call rate was <95%, or SNPs
193 presenting a significant departure from Hardy-Weinberg equilibrium ($P < 0.05$ following the
194 Chi-squared test), were excluded from all subsequent analysis (336 SNPs excluded leaving
195 1785 SNPs for the PLS regression analysis).

196

197 *Statistics - Partial least squares regression*

198 In order to identify SNPs associated with the variability in the postprandial CM LUT
199 response, we employed partial least squares regression (PLS). PLS is a multivariate statistical
200 tool often used for chemometric (38) and spectrometric modeling, and which has recently
201 been applied to SNP-based predictions by our group (31) and others (19, 31, 39, 40). Due to
202 the large number of SNPs compared with the low number of subjects and multicollinearity
203 between SNPs, PLS regression was chosen to identify SNPs (among the 1785 candidate
204 SNPs) that were predictive of the postprandial CM LUT response, according to their variable
205 importance in the projection (VIP) value. A general genetic model was assumed, *i.e.* the three
206 genotypes of each SNP were treated as separate categories with no assumption made about
207 the effect conferred by the variant allele for homozygotes or heterozygotes on the postprandial
208 CM LUT response. Different PLS regression models were built using increasing VIP
209 threshold values. The model maximizing the explained (R^2) and the predicted variance (Q^2)
210 and validated following cross-validation ANOVA was selected. Additional validation
211 procedures of the PLS regression models (41, 42) were also performed and are described in
212 **Supplementary Methods**. Simca-P12 software (Umetrics, Umeå, Sweden) was used for all
213 multivariate data analyses and modeling.

214

215 *Statistics - Univariate analysis*

216 In a second approach, we performed univariate analyses to compare the postprandial
217 CM LUT response between subgroups of subjects who bore different genotypes for the SNPs
218 selected in the PLS regression model. Differences obtained in the different genotype
219 subgroups were analyzed using Student's t-test with the Benjamini-Hochberg correction,
220 using QVALUE software (43). For all tests, a false discovery rate q-value <0.05 was
221 considered significant.

222

223 **Results**

224 *Interindividual variability in CM LUT responses to the meals*

225 **Figure 1** shows the postprandial CM LUT response after consumption of the meal
226 containing the LUT supplement. The CV of the postprandial CM LUT response, called “LUT
227 response” hereafter in the manuscript, was 75%, as illustrated in **Figure 2**. Lower LUT
228 responses with a higher variability (CV=137%) were observed after consumption of the
229 tomato-sourced LUT meal (n=33 of the 39 subjects)(data not shown).

230

231 *Correlation between the LUT responses measured after consumption of the two meals*

232 In order to assess whether the ability to respond to dietary LUT was an intrinsic
233 characteristic of the subjects, we calculated the correlation between the LUT responses
234 measured after the two meals. Results showed that there was a significant positive relationship
235 (Pearson’s $r=0.68$, $P<0.0001$ and Spearman’s rank correlation coefficient= 0.45 , $P=0.011$)
236 between the two responses.

237

238 *Correlations between the LUT responses to the meals and the fasting plasma LUT* 239 *concentrations*

240 In order to assess whether the fasting plasma concentration of LUT, which is known to
241 be a marker of LUT status, was related to the ability to respond to dietary LUT, we calculated
242 the correlation between the LUT response of the subjects and their fasting plasma LUT
243 concentration. To estimate the fasting plasma LUT concentration, we averaged four fasting
244 plasma LUT concentrations measured at least 3 weeks apart. Results showed that there was a
245 significant positive relationship between the LUT response to the meal that contained the LUT
246 supplement and the fasting plasma LUT concentration (Pearson’s $r=0.51$, $P<0.003$ and
247 Spearman’s rank correlation coefficient= 0.49 , $P=0.006$). The relationship between the LUT

248 response to the meal that contained tomato puree, as a source of LUT, and the fasting plasma
249 LUT concentration was also positive (Pearson's $r=0.3$ and Spearman's $\rho=0.3$), although non
250 significant ($P=0.09$ and $P=0.07$ respectively), likely because of the higher variability of the
251 LUT response observed after this meal (see above).

252

253 *Genetic variants associated with the LUT response*

254 As stated previously, we used PLS regression to examine whether the 1785 candidate
255 SNPs (used as qualitative X variables) could explain a significant part of the variability in the
256 LUT response of the 39 subjects following consumption of the meal containing the LUT
257 supplement. We focused on the the LUT supplement-containing meal because of the higher
258 number of subjects and the higher, more accurate LUT response measured, due to the higher
259 dose of LUT in this meal. As shown in **Table 2**, the model including all SNPs described the
260 group variance with good accuracy ($R^2=0.92$) but was not predictive of this variance ($Q^2=-$
261 0.10). Therefore, to improve the model and find an association of SNPs more predictive of the
262 LUT response, we filtered out SNPs that made no important contribution, *i.e.* those who
263 displayed the lowest variable importance in the projection (VIP) value. After applying several
264 thresholds of VIP value (**Table 2**), we found that the best model obtained included 39 SNPs
265 (29 not in linkage disequilibrium plus 10 in linkage disequilibrium which are shown in
266 **Supplementary table 3**). The 29 SNPs were located in or near 15 genes (**Table 3**) and
267 described 73 % of the group variance, with a prediction index Q^2 of 56 % (**Table 2**). The
268 robustness and the stability of the model were validated by three additional methods detailed
269 in the **Supplementary Methods** section.

270 Using univariate statistics, the association of the 29 SNPs with the LUT response was
271 further evaluated by comparing for each SNP the LUT response of subjects who bore different

272 genotypes (**Table 3**). For 11 of the 29 SNPs, subjects bearing different genotypes exhibited a
273 significantly different LUT response (q-value<0.05).

274

275 *Genetic score to predict the LUT response of a genotyped subject*

276 Knowing a subject's genotype at the 29 aforementioned loci, it is possible to predict
277 his ability to respond to LUT according to the following equation:

$$\widehat{RP} = a + \sum_1^{29} r_i * genotype.(SNP_i)$$

278 With RP as the responder phenotype (i.e. the LUT response), a as a constant, r_i as the
279 regression coefficient of the i^{th} SNP included in the PLS regression model, and
280 $genotype.(SNP_i)$ as a Boolean variable indicating the subject's genotype at the i^{th} SNP. A list
281 of the regression coefficients calculated by the SIMCA software can be found in
282 **Supplementary table 4.**

283

284 **Discussion**

285 The first noteworthy observation was the high interindividual variability observed in
286 the LUT response to the two LUT-containing meals (CV of 75% and 137% after the meal
287 providing the LUT supplement and the tomato meal, respectively). This is in agreement with
288 the high interindividual variability reported for β -carotene (44-48), lycopene (48) and LUT
289 (48). The higher interindividual variability observed after intake of the tomato meal compared
290 to the meal containing the LUT supplement was likely due to the lower amount of LUT
291 provided by the tomato puree (0.1 vs 15 mg), which likely led to a lesser accuracy in the CM
292 LUT measure. Nevertheless, several other additional factors could also participate in this
293 higher interindividual variability: i) the lower number of subjects who consumed the tomato
294 meal as compared to the LUT supplement meal (33 instead of 39), ii) the tomato matrix in
295 which LUT was embedded and which contained fibers that may have impaired LUT
296 absorption (49) iii) a competition with other carotenoids present in the tomato matrix, e.g.
297 lycopene and β -carotene (34), iii) a higher variability in gastric emptying rate due to the 100 g
298 tomato puree, iv) a higher variability in CM clearance rates.

299 The second noteworthy observation was that the LUT responses measured after the
300 two meals in the same subjects were significantly correlated. This suggests that, as previously
301 observed for β -carotene (44, 45, 48), the ability to absorb dietary LUT is an intrinsic, likely
302 genetic, characteristic of the subjects. This, together with the fact that several proteins have
303 been associated with LUT absorption and transport within the body (25), led us to hypothesize
304 that polymorphisms in genes involved in LUT metabolism might be involved in the LUT
305 response (18, 50). Since the LUT response is a complex phenotype that likely involves several
306 genes, a thorough study of the association between this phenotype and candidate genetic
307 variants should simultaneously include SNPs in all genes that are assumed to be involved in
308 this phenotype. The results of the multivariate analysis, whose validity was checked by several

309 tests (see **Subjects and Methods** section as well as the **Supplementary Methods** section),
310 showed that a significant part (73%) of the interindividual variability in the LUT response
311 could be assigned to 29 SNPs in or near 15 genes, out of 51 candidate genes. Seven out of
312 these 29 SNPs (**Table 3**) have recently been found to be involved in the postprandial CM
313 triacylglycerol response in the same group of subjects (31). This observation is not surprising
314 as it is assumed that most newly absorbed LUT is carried from the intestine to peripheral
315 organs via CM, which are mainly constituted of triacylglycerols. Because this study aims to
316 identify genes that are specifically associated with the LUT response, we will only discuss the
317 potential role of the four genes that had not been associated with the CM triacylglycerol
318 response (31) and which displayed a significant *P*-value following univariate statistics (**Table**
319 **3**), *i.e.* *ISX* (Intestine Specific Homeobox), *ELOVL2* (ELOVL fatty acid elongase 2), *ABCG2*
320 (ATP-binding cassette, sub-family G, member 2) and *MTTP*. First, it is striking to observe that
321 the SNPs in these four genes were the most important in the PLS model, *i.e.* they had the
322 highest VIP value were thus the main contributors in LUT variability. *ISX* has been shown to
323 act as a transcriptional repressor of SR-BI expression in the intestine (51). Because SR-BI is
324 involved in LUT uptake (22), we hypothesize that SNPs in *ISX* may affect its
325 expression/activity. This in turn would affect SR-BI expression and thus LUT uptake
326 efficiency. *ABCG2* encodes for a breast cancer resistance protein (BCRP) which is a
327 multidrug transporter (52). As far as we know, there is currently no study on the involvement
328 of this protein in LUT transport. However, we selected it as a candidate gene because
329 flavonoids have been shown to be inhibitors of *ABCG2/BCRP* (53) and the flavonoid
330 naringenin impairs LUT uptake in Caco-2 cells (54). Thus, the association of SNPs in
331 *ABCG2/BCRP* with the LUT response suggests that this protein participates in LUT
332 absorption. Further studies are required to verify this hypothesis. *MTTP* encodes for the
333 microsomal triglyceride transfer protein, which is involved in the packaging of

334 triacylglycerols within the CM. The association between a SNP in *MTTP* and the LUT
335 response is therefore not surprising, as LUT is incorporated into CM in the enterocyte.
336 *ELOVL2* catalyzes the elongation of EPA to DPA and, subsequently, DHA. This association
337 was also observed in a previous study where the rs1150561 SNP near *ELOVL2* was associated
338 with blood LUT response to LUT supplementation (16). The mechanism of this relationship is
339 uncertain and requires further study.

340 The third noteworthy observation was that the subjects' fasting plasma LUT
341 concentrations and their LUT responses were positively correlated. This relationship has been
342 previously reported by Norkus *et al.* (55) who observed that higher initial (baseline) serum
343 LUT concentrations predicted a greater serum LUT response after LUT supplementation. This
344 suggests that the ability to respond to dietary LUT is a key factor that governs blood, and
345 likely tissue, LUT concentration.

346

347 To conclude, results of this study demonstrate that the interindividual variability in
348 LUT bioavailability is at least partially genetically controlled. They also show that the ability
349 to absorb dietary LUT is an important determinant of circulating fasting blood concentrations
350 of LUT. Finally, our results suggest that a significant portion of the interindividual variability
351 in LUT bioavailability can be explained by a combination of SNPs, most of them located in or
352 near genes that have been associated with CM triacylglycerol response. We believe that this
353 study will be the starting point of a series of studies aiming to genetically predict LUT
354 bioavailability among individuals or more probably group of individuals carrying key SNPs
355 involved in LUT bioavailability. The objective of these kinds of studies will be to give
356 nutritionists an accurate and validated genetic tool to predict LUT response for future
357 supplementation studies. Ultimately, this approach could be used to help optimize LUT intake

358 for those who may be at risk for developing diseases which may benefit from LUT

359 consumption (i.e. macular degeneration, cognitive decline, etc.).

360

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363

364 **Authors' contributions to manuscript:**

365 PB designed research; MN, NL and SM conducted the clinical research; RB analysed lutein
366 by HPLC; CD and PB analyzed data and CD performed statistical analysis; PB and CD wrote
367 the paper; PB and CD had primary responsibility for final content.

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Tables

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

Age (y)	32.3 ± 2.0 ¹
Weight (kg)	73.5 ± 1.3
BMI (kg/m ²)	23.0 ± 0.3
Glucose (mmol/L) ²	4.8 ± 0.1
Triacylglycerol (g/L) ²	0.7 ± 0.1
Total cholesterol (g/L) ²	1.6 ± 0.1
Hemoglobin (g/dL) ²	15.1 ± 0.1
Lutein (µmol/L) ^{2,3}	0.30 ± 0.01
Postprandial chylomicron lutein response (nmol/L/h) ⁴	69.0 ± 8.3

¹ Mean ± SEM, n=39.

² Fasting plasma parameters.

³ Since the fasting plasma lutein concentration can be affected by recent dietary intakes of lutein, blood levels were measured on four occasions for each subject, with at least a 3 week interval between each measurement.

⁴ Incremental area under the curve of chylomicron lutein concentration measured during the postprandial period (0 to 8 h) following the intake of the meal providing the lutein supplement.

Table 2. Performances of the different partial least square regression models that explained the postprandial chylomicron lutein response to the meal containing the lutein supplement.¹

VIP threshold	R ²	Q ²	SNP number	<i>P</i> (CV-ANOVA)
No selection	0.92	-0.10	1785	1
VIP>0.5	1.00	0.59	505	0.99
VIP>1.0	0.95	0.61	148	0.14
VIP>1.5	0.76	0.49	69	2.7 x 10 ⁻³
VIP>1.6	0.74	0.50	62	2.2 x 10 ⁻³
VIP>1.7	0.73	0.47	55	2.2 x 10 ⁻³
VIP>1.8	0.73	0.56	39	1.9 x 10⁻⁴
VIP>1.9	0.71	0.52	31	1.8 x 10 ⁻⁴
VIP>2.0	0.69	0.52	26	1.2 x 10 ⁻⁴
VIP>2.1	0.61	0.47	18	2.0 x 10 ⁻⁴
VIP>2.2	0.62	0.48	17	1.2 x 10 ⁻⁴
VIP>2.3	0.58	0.46	13	3.1 x 10 ⁻⁴
VIP>2.4	0.58	0.46	13	3.1 x 10 ⁻⁴

VIP>2.5	0.54	0.42	11	7.0×10^{-4}
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¹ Different partial least square 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 **Supplemental Material**), was selected. This is the model with VIP >1.8, shown in bold font.

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

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

Gene and SNP rs number ²	VIP value	SNP minor allele frequency	q-value ³
<i>ELOVL2</i> -rs9468304	3.37	0.309	0.020
<i>ABCG2</i> -rs17731631	3.20	0.125	0.020
<i>ELOVL2</i> -rs3798709	3.19	0.250	0.025
<i>ISX</i> -rs137252	2.84	0.090	0.020
<i>MTTP</i> -rs17029213	2.65	0.051	0.003
<i>ISX</i> -rs137269	2.62	0.132	0.033
<i>LPL</i> -rs1441778	2.60	0.189	0.063
<i>APOA1</i> -rs2070665	2.50	0.180	0.020
<i>ABCG2</i> -rs6532059	2.41	0.047	0.026
<i>ISX</i> -rs5749706	2.25	0.199	0.068
<i>MC4R</i> -rs11873337	2.21	0.128	0.092
<i>ISX</i> -rs137238	2.17	0.122	0.092
<i>PKDIL2</i> -rs12596941	2.09	0.203	0.092
<i>INSIG2</i> -rs17006621	2.09	0.203	0.092
<i>IRS1</i> -rs2178704	2.06	0.199	0.093
<i>LPL</i> -rs7821631	2.05	0.204	0.092
<i>APOB</i> -rs2854725	2.03	0.092	0.063
<i>LPL</i> -rs10096561	2.02	0.146	0.102
<i>ABCA1</i> -rs4149316	2.01	0.117	0.020
<i>RPE65</i> -rs1924546	2.00	0.119	0.078
<i>ABCA1</i> -rs390253	1.96	0.257	0.094
<i>LPL</i> -rs256	1.94	0.140	0.102

<i>LIPC</i> -rs12591216	1.91	0.096	0.094
<i>ABCA1</i> -rs9919066	1.89	0.063	0.094
<i>ABCA1</i> -rs2020926	1.88	0.105	0.003
<i>COBLL1</i> -rs3769877	1.82	0.189	0.102
<i>IRS1</i> -rs1316328	1.81	0.142	0.033
<i>ISX</i> -rs5755368	1.81	0.234	0.124
<i>LIPC</i> -rs12593880	1.80	0.075	0.094

¹ SNPs present in the selected partial least square regression model shown in **Table 2**. Note that 18 out of the 39 SNPs present in the selected model were in linkage disequilibrium. Since these SNPs provided redundant information in the model, 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**.

² SNPs are ranked by decreasing VIP value.

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

Abbreviations: 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 **Supplemental table 1**.

Figure Legends

Figure 1: Incremental chylomicron lutein concentrations measured after consumption of the meal containing the lutein supplement.

For each subject, postprandial chylomicron lutein concentrations were baseline-corrected using the fasting chylomicron lutein concentration. Bold curve: mean \pm SEM values of the 39 subjects. Lower dotted curve: concentration of lutein measured in the lowest responder, *i.e.* the subject who had the lowest area under the curve of the postprandial chylomicron lutein concentration among all the subjects. Higher dotted curve: concentration of lutein measured in the highest responder.

Figure 2: Individual postprandial chylomicron lutein responses after consumption of the meal containing the lutein supplement.

Subjects were sorted by increasing postprandial chylomicron lutein response, *i.e.* 0-8 h area under the curve.