

Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans

Patrick Borel, Fabien Szabo de Edelenyi, Stéphanie Vincent-Baudry, Christiane Malezet-Desmoulin, Alain Margotat, Bernard Lyan, Jean-Marie Gorrard, Nathalie Meunier, Sophie Drouault-Holowacz, Severine Bieuvelet

► **To cite this version:**

Patrick Borel, Fabien Szabo de Edelenyi, Stéphanie Vincent-Baudry, Christiane Malezet-Desmoulin, Alain Margotat, et al.. Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations and macular pigment optical density in humans. *Annals of Medicine*, Taylor & Francis, 2011, 43 (1), pp.47 - 59. 10.3109/07853890.2010.531757 . inserm-01478670

HAL Id: inserm-01478670

<https://www.hal.inserm.fr/inserm-01478670>

Submitted on 20 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Genetic variants in BCMO1 and CD36 are associated with plasma lutein concentrations**
2 **and macular pigment optical density in humans.**

3

4 **Running title:** Genes involved in plasma lutein and macular pigment

5

6 Patrick Borel^{1,2,3}, Fabien Szabo de Edelenyi^{1,2,3}, Stéphanie Vincent-Baudry⁴, C. Malezet-
7 Desmoulin^{1,2,3}, Alain Margotat^{1,2,3}, Bernard Lyan⁵, Jean-Marie Gorrard⁶, Nathalie Meunier⁵,
8 Sophie Drouault-Holowacz⁴, Severine Bieuvelet⁴

9

10 ¹ INRA, UMR1260 «Nutriments Lipidiques et Prévention des Maladies Métaboliques», F-
11 13385 Marseille, France

12 ² INSERM, ERL U1025, F-13385 Marseille, France

13 ³ Univ Aix-Marseille 1, Univ Aix-Marseille 2, F-13385 Marseille, France

14 ⁴ Pileje, 75015 Paris, France

15 ⁵ Centre de Recherche en Nutrition Humaine d'Auvergne, 58 rue Montalembert, 63009
16 Clermont Ferrand Cédex, France

17 ⁶ Laboratoire de Biophysique sensorielle, Faculté de Médecine, 63001 Clermont-Ferrand,
18 France

19

20 Corresponding author:

21 Patrick BOREL, Ph.D.

22 UMR 1260 INRA / ERL U1025 INSERM -NLPMM

23 Faculté de Médecine, 27 Boulevard Jean-Moulin, 13385 Marseille Cedex 5, FRANCE.

24 E-mail: Patrick.Borel@univ-amu.fr

25

26 **Abstract**

27

28 Lutein is recovered at high concentration in the human macula lutea. Recent studies suggest
29 that this micronutrient might be implicated in prevention of age-related macular degeneration.

30 **Objective:** to identify genes which affect blood and retina lutein concentrations among

31 candidate genes (intestinal sterol transporters and carotenoid oxygenases). **Design:** a

32 comparative plus an observational study. **Participants:** Twenty nine healthy subjects for the

33 comparative study and 622 subjects for the observational study. **Intervention and methods:**

34 All the participants were genotyped for single nucleotide polymorphisms (SNPs) in the

35 candidate genes. Fasting plasma lutein concentrations were measured in all the participants

36 and after six months supplementation, with either a lutein-rich supplement or a placebo, in the

37 29 subjects who participated in the comparative study. Macular pigment optical density

38 (MPOD), which is a measure of macula concentration of lutein, was measured before and

39 after the dietary intervention in the 29 subjects. Associations between SNPs and plasma lutein

40 and MPOD were assessed by Partial Least Square (PLS) regression followed by univariate

41 analysis. Observed associations between SNPs and plasma lutein were verified by haplotype-

42 based associations analysis in the cohort of 622 subjects. **Main outcome measures:** plasma

43 lutein levels and MPOD. **Results:** Six SNPs in four genes (ABCG8, BCMO1, CD36 and

44 NPC1L1) explained 25% and 38% of the plasma and MPOD variance, respectively. Subjects

45 with TT at the BCMO1 rs7501331 locus had lower ($P < 0.05$) plasma lutein than CT subjects.

46 Subjects with CC at the CD36 rs13230419 locus had lower ($P < 0.05$) plasma lutein than

47 subjects who carried a T allele. The association between CD36 and plasma lutein was

48 confirmed in the cohort of 622 subjects. Subjects with TT at the BCMO1 rs7501331 locus had

49 a higher ($P < 0.05$) MPOD, and subjects with GG at rs1761667 CD36 locus had a higher ($P <$

50 0.05) MPOD than those with an A allele. **Conclusions:** These results suggest that BCMO1

51 and CD36 are implicated in plasma and retina concentrations of lutein and that genetic
52 variants in these genes can modulate blood and retina concentrations of lutein.

53

54 **Key words:** age-related macular degeneration, bioavailability, carotenoids, eye, genetic
55 variant, genetic polymorphisms, nutrition, retina, xanthophylls

56

57 **Key messages:** Age-related macular degeneration is an degenerative eye disease that is due, at
58 least in part, to free radicals, and several studies have suggested that antioxidant
59 micronutrients, such as vitamin C, E, carotenoids, and selenium, may participate in the
60 defense of the retina against free radicals. The main carotenoids found in the human retina are
61 the xanthophylls, lutein, zeaxanthin and meso-zeaxanthin. Our results suggest that genetic
62 variants in BCMO1 and CD36 modulate plasma and retina lutein concentrations.

63

64 **Abbreviations:**

65

66 BCMO1: β -carotene monooxygenase 1

67 BCDO2: β -carotene dioxygenase 2

68 BMI: body mass index

69 MPOD: macular pigment optical density

70 HPLC: high pressure liquid chromatography

71 PLS: partial least square regression

72 SNP: single nucleotide polymorphisms

73

74 **Introduction**

75

76 Age-related macular degeneration is an degenerative eye disease that is due, at least in
77 part, to free radicals (1). Several studies have suggested that antioxidant micronutrients, such
78 as vitamin C, E, carotenoids, and selenium, may participate in the defense of the retina against
79 free radicals. The main carotenoids found in the human retina are the xanthophylls, lutein,
80 zeaxanthin and meso-zeaxanthin (2, 3). Macular meso-zeaxanthin apparently originates from
81 the metabolism of lutein (4), while lutein and zeaxanthin originate from the diet. Lutein and
82 zeaxanthin are mainly obtained from fruits and vegetables. It is assumed that carotenoids are
83 extracted from plant cells in the upper part of the gastrointestinal tract (5, 6), solubilized in fat
84 lipid droplets (7) and incorporated into mixed micelles (8). Mixed micelles are assumed to
85 carry carotenoids to the intestinal brush border where they are absorbed by the enterocytes (9).

86 Mechanisms involved in intestinal absorption of carotenoids were initially studied by
87 the Hollander group (10, 11). This group concluded that the intestinal absorption of beta-
88 carotene, and, by extension, of all the carotenoids, is passive (12). However, this dogma was
89 refuted by recent studies that have shown that absorption of several carotenoids (13-17)
90 involves an enterocyte apical membrane protein, SR-BI (scavenger receptor class B type I),
91 which has been involved in cholesterol uptake. Interestingly, the result obtained in the study
92 dedicated to lutein absorption suggested that other transporters are probably involved (13). We
93 hypothesized that these transporters may be sterol transporters because of the involvement of
94 SR-BI and because they have low substrate specificity. At present, there are several sterol
95 transporters that have been identified: 1) NPC1L1, which is apparently the main protein
96 involved in cholesterol uptake (18) and which has recently been found to be involved in
97 vitamin E uptake (19); 2) SR-BI, which is involved in the uptake of cholesterol (14),
98 carotenoids (13, 14, 17) and vitamin E (20); 3) ABCG5 and ABCG8, which are involved in

99 phytosterols and cholesterol efflux back into the intestinal lumen (21) and 4) ABCA1, which
100 is mainly located at the basolateral side of the enterocyte and is involved in cholesterol (22)
101 and tocopherol efflux (23, 24).

102 It is assumed that, after absorption, lutein is incorporated into chylomicrons and
103 transported to the liver. A fraction of lutein is then incorporated into VLDL and distributed to
104 peripheral tissues by lipoproteins (25-27). A recent study has suggested that SR-BI is involved
105 in xanthophyll uptake by retina cells (28). Since the sterol transporters mentioned above are
106 expressed not only in the intestine but also in various other tissues, we hypothesized that they
107 are good candidates for retina uptake of lutein. Finally, it is assumed that BCMO1 (β -carotene
108 monooxygenase 1) and/or BCDO2 (β -carotene dioxygenase 2), which are involved in cleavage
109 of provitamin A carotenoids in retinal and apo-carotenals, respectively (29), might be
110 involved in the metabolism of lutein and thus in its blood and tissue concentration. This is
111 supported by a recent study showing that a nonsense mutation in BCDO2 was associated with
112 the yellow skin phenotype in sheep, suggesting a broad specificity of this enzyme for
113 carotenoids (30).

114

115 The main objective of the present study was to assess whether some sterol transporters
116 and carotene oxygenases are involved in blood and macular concentrations of lutein. To attain
117 our goal, we studied associations between single nucleotide polymorphisms (SNPs) of genes
118 that encode these transporters and the two carotene oxygenases, and blood and macula
119 concentrations of lutein in 29 healthy subjects. Observed associations between genetic
120 variants and plasma lutein were further verified in a cohort of 622 subjects.

121

122 **Material and Methods**

123

124 *Subject number and characteristics*

125 Since there were no data on the effect of the selected genetic variants on either plasma
126 lutein levels or macular pigment optical density (MPOD), we were unable to perform a power
127 analysis to calculate the number of subjects required to observe a significant effect with an
128 80% power. We decided to work with 30 subjects in order to divide the group into two
129 subgroups with 15 subjects each: one took a lutein supplement; the other, a placebo (see the
130 paragraph on lutein supplementation).

131 Thirty healthy, non-obese males were recruited. They had no disease history,
132 hyperlipemia or hyperglycemia. Their characteristics and daily nutrient intakes are reported in
133 **Table 1**. One subject withdrew for personal reasons during the study. Twenty-nine of the
134 thirty selected subjects were non-smokers. They were not taking any medication known to
135 affect lutein or lipid metabolism during the month before the study started or during the study
136 period. The study was approved by the regional committee on human experimentation (CPP
137 Sud Est VI, France) and adhered to the tenets of the Declaration of Helsinki. The objectives
138 and requirements of the study were fully explained to the participants, and informed written
139 consent was obtained for each subject. The subjects' usual diet was estimated with a three-day
140 food diary before the study started. Portion sizes were estimated with photographs compiled in
141 a manual adapted from the Su.Vi.Max picture booklet (31). The dietary diary was analyzed for
142 nutrient composition with a diet analyzer software (GENI 6.5; Micro6, Villers les Nancy,
143 France). The software database was extended for carotenoids by mean of the USDA
144 carotenoid food-composition database (32). The lutein intake was close to that previously
145 observed in French group of volunteers (33-35), and close to the intake reported in a US
146 population (36).

147

148 *Choice of candidate SNPs*

149 Candidate SNPs in genes involved in sterol absorption were selected through an
150 analysis of previous studies describing associations between these SNPs and digestion,
151 transport or metabolism of sterols. The SNP in BCDO2 was advised by Dr George Lietz
152 (Newcastle University, UK) from an unpublished study. Characteristics of the SNPs are
153 presented in **Table 2**. SNPs were validated for the oligo-ligation assay (SNPlex, see below)
154 by several criteria: 1) genome screening, in which the SNPs may be located in a genome
155 region that is homologous with at least one other genome region, leading to a lack of assay
156 specificity and the potential for spurious ligation templates; 2) assay rules, in which an
157 individual SNP assay cannot be designed due to deleterious sequence contexts or non optimal
158 interactions among the assay components; for example, aspects of the SNP sequence or assay
159 components may result in secondary structure and reduce assay performance, including a
160 series of contiguous Gs or a series of 16 weak contiguous bases (A's or T's) within 25 bases
161 of the SNP and 3) pooling rules, in which deleterious potential interactions may occur
162 between specific SNP assays in the assay pools and false signals may be generated due to
163 components from different assays interacting with genomic DNA.

164 The SNPs that were not validated for SNPlex were replaced by alternate SNPs, in
165 linkage disequilibrium with the initial SNPs, or analyzed by Taqman (see thereafter).

166

167 *DNA preparation and genotyping methods*

168 Genomic DNA was prepared from 2 ml whole blood and purified with the
169 NucleoSpin® Blood L kit ref 740 954 (Macherey Nagel, Hoerd, France). A mean of 15 µg of
170 DNA was isolated from each blood sample. The purity and quantity of DNA was checked by
171 spectrophotometry at 260 nm and 280 nm. A total of 100 µl of DNA at a concentration of 10

172 ng/μl was added to a plate (Dutscher, Marseille, France) for genotyping. SNPs were
173 genotyped with an oligo-ligation assay (SNPlex, Applied Biosystems, Foster City)(37, 38) or
174 a TaqMan method (Applied Biosystems) following the manufacturers' guidelines.

175 The oligo-ligation assay consists of designing 3' specific primers for each SNP, with
176 two primers carrying the SNP-base specific 3' end and one common primer that starts 5' to
177 the next base in the target sequence. The two allele specific primers carry unique ZIP codes
178 that determine each allele. Primers are annealed to the target sequence according to the
179 manufacturer's recommendations. A ligation reaction will join the allele specific primer with
180 the common primer if the allele specific 3'-base is present. A short fluorescent dye labeled
181 probe, homologous to the ZIP code sequence, is then hybridized to the immobilized product.
182 Up to 48 SNPs can thus be multiplexed into one oligo-ligation reaction. Following the
183 manufacturer's recommendations, genomic DNA was heat fragmented. The allele specific
184 fluorescent probes were separated on an automated sequencer (ABI 3730, Applied
185 Biosystems, Foster City). Alleles were binned and called with the GeneMapper software
186 (Applied Biosystems, Foster City).

187 The TaqMan assays were performed when selected SNPs could not be analyzed by
188 SNPlex. This was the case for two SCARB1 SNPs, rs4238001, and the one called "intron 5".
189 Probes were purchased from the manufacturer and used according to the manufacturer's
190 guidelines. DNA was PCR-amplified by denaturation at 95°C for 10 min, 40 cycles at 92°C
191 for 15 sec, 60°C for 1 min, and 72°C for 45 sec, followed by elongation at 72°C for 5 min.
192 TaqMan assays were then read on a 7900HT Fast Real-Time PCR System (Applied
193 Biosystems), and alleles were called by the SDS software (Applied Biosystems).

194

195

196

197 *Lutein supplementation*

198 The 29 subjects were first asked to follow a lutein-poor diet for 3 wks. To attain this
199 objective they were asked to discard lutein-rich foods from their usual diet (a list of lutein-rich
200 food was given to the volunteers). The subjects then came to a Center for Clinical
201 Investigation (Centre de Recherche en Nutrition Humaine d’Auvergne) after an overnight fast,
202 and a blood sample was collected. The same day, the subjects also came to a biophysics
203 laboratory to have their MPOD measured (the right eye was used for most of the subjects).
204 Subjects were randomly assigned to one of the two groups. One received a placebo, while the
205 other received a lutein-rich supplement (Visiobane Protect, Pileje, France) for 6 months. The
206 supplement was provided as two pills containing 5 mg of lutein esters each. Subjects were
207 asked to eat the pills during their main meals. The supplement also contains : Porphyra, B
208 vitamins, vitamin C and E, fish oil, bee wax and gelatine. The placebo contained only refined
209 sunflower oil. At the end of the supplementation period, all the subjects came back to the
210 Center for Clinical Investigation for collection of a second fasting blood sample, and to the
211 biophysics laboratory for another MPOD measurement.

212

213 *Measurement of macular pigment optical density (MPOD)*

214 The MPOD was determined as described by the van Norren team (39, 40). In
215 summary, the radiant flux of the pattern radiated from photoreceptors at the fovea (sample
216 field: 2 deg) and the radiant flux of the beam reflected specularly by the ILM in the perifovea
217 (used as reference) were measured. The measuring light was provided by a 75W Xenon lamp
218 (Oriol) that was filtered either by an interference filter at 470 nm (FWHM 10 nm, Oriol), or an
219 interference filter at 532 nm (FWHM 10 nm, Oriol). The retinal illuminances were 4.8 logTd
220 and 5.5 logTd for the blue and green lights, respectively. The distribution of light in the pupil
221 was measured with a CCD camera cooled by liquid nitrogen (Princeton Instruments). This

222 camera had a resolution of 512x512 pixels, with an image depth of 16 bits. The pixel size in
223 the pupil was 0.0255 mm. We combined areas of 2x2 pixels, giving a resolution of 0.051
224 mm/pixel. Two stepping motors X and Y (Newport) allowed the center J of the entrance pupil
225 (dia 0.2 mm) to be positioned at the chosen location in the eye's pupil. The head of the subject
226 was stabilised by a bite bar which was fixed on a three-dimensional positioner. The main
227 optical components were mounted on a single plate which could be shifted longitudinally,
228 thus allowing focus adjustment from -12 diopters to +12 diopters of ametropia. The pupil was
229 dilated by application of 0.5% Mydriacyl to a minimum diameter of 7 mm. The eye was then
230 aligned to the reflectometer. A fixation target was used to direct the subject's gaze either to
231 the center of the sampling area or at a site 6 deg temporal to the fovea. At the fovea the center
232 J of the entrance pupil was aligned to photoreceptor axes. At 6 deg eccentricity, the point J
233 was positioned at the center of the eye's pupil. For each of these retinal eccentricities, the
234 measuring green beam bleached the retina for a period of 15 s, then three pupil images were
235 captured at 532 nm (integration time of 4 s), with an interval of 10 s between each one; they
236 were followed by three pupil images at 470 nm.

237

238 *Plasma lutein extraction and HPLC analysis of lutein*

239 Plasma lutein was extracted and analyzed as previously described (41). Briefly, 200
240 μL of plasma was deproteinized by adding one volume of ethanol containing the carotenoid
241 echinenone (as an internal standard). Lutein was extracted twice by the addition of two
242 volumes of hexane. The hexane phases obtained after centrifugation (500 g, 10 min, 4°C)
243 were pooled and evaporated completely under nitrogen. The dried extract was dissolved in
244 200 μL of dichloromethane/methanol mixture (65/35; V/V). All extractions were performed at
245 room temperature under yellow light to minimize light-induced damage. A volume of 80 μL
246 was used for HPLC analysis. The HPLC system consisted of a 150 X 4.6 mm, RP C₁₈, 3- μm

247 Nucleosil column (Interchim, Montluçon, France) coupled with a 250 X 4.6 mm RP C₁₈, 5-
248 µm VydacTP 54 column (Hesperia, CA) and a 10 X 4.6 mm RP C₁₈, 5-µm hypersil guard
249 column. The mobile phase consisted of acetonitrile/methanol containing 50 mmol/L
250 ammonium acetate/water/dichloromethane (70/15/5/10; V/V/V/V). Solvents were HPLC
251 grade from Carlo Erba - SDS (Peypin, France). The flow rate was 2 mL/min. The columns
252 were kept at a constant temperature (30°C). The HPLC system consisted of a Waters system
253 equipped with a UV-visible photodiode-array detector (Waters 996). Carotenoids were
254 detected at 450 nm and identified by their retention time compared with pure (> 95%)
255 standards. Quantifications were performed with Millennium 32 software (version 3.05.01),
256 comparing peak area with carotenoid standard reference curves. Carotenoid standards were a
257 generous gift of DSM LTD, Basel, Switzerland.

258

259 *Plasma lipids and apolipoproteins*

260 Triglyceride and total cholesterol concentrations were determined by enzymatic
261 procedures with commercial kits (Roche, Switzerland). High-density lipoprotein (HDL)
262 cholesterol was measured after sodium phosphotungstate–magnesium chloride precipitation.
263 Low-density lipoprotein (LDL) cholesterol was estimated indirectly by use of the Friedewald
264 formula.

265

266 *Characteristics of the cohort of 622 subjects*

267 In order to verify the associations observed in the study on 29 volunteers we decided
268 to verify associations observed between genetic variants and plasma lutein on a cohort of 622
269 french subjects. This cohort was a subset of the french SUVIMAX cohort (42). Characteristics
270 of this cohort were as follow: 281 males and 341 females, 61.65 ± 0.25 years old, BMI: 25.76
271 ± 0.17 Kg/m², plasma cholesterol: 2.23 ± 0.01 g/l, plasma lutein: 540 ± 10 nmol/L. Subjects

272 were genotyped for SNPs in CD36 and BCMO1 and haplotype effects of these genes on
273 plasma lutein were tested.

274

275 *Statistical analysis*

276 As a first approach, we wanted to estimate the relationships between genotypes, and
277 both plasma lutein and MPOD in the study on 29 volunteers. For this purpose, we used partial
278 least square (PLS) regression. PLS regression was performed by the SIMCA-P software,
279 version 11.0 (Umetrics, Umeå, Sweden). The Y matrix was composed of the 2 vectors
280 containing the initial plasma lutein concentrations and the initial MPOD. The X matrix
281 contained the genotypes for the 20 SNPs. The values in the Y matrix were scaled with a unit
282 variance scaling prior to the calculation of the latent vectors. After examination of the
283 importance of each dependent variable, it appeared that only six SNPs were significant for the
284 regression model. Therefore, a second regression, which used the genotypes from these six
285 SNPs in the X matrix, was performed in order to minimize the noise in the model. The PLS
286 model was calculated with the function “autofit” in the SIMCA-P software in order to find the
287 optimal number of latent vectors. Note that correction for multiple testing was not compulsory
288 with PLS regression because independent variables are not treated independently but are used
289 simultaneously to find a reduced number of latent variables for the model. Furthermore,
290 model validation, testing for the robustness of the model and its generalization to new data, is
291 performed in this model with cross-validation.

292 In a second approach, we performed univariate statistical analyses only in associations
293 that were simultaneously significantly associated with the two independent markers of lutein
294 metabolism, which were plasma lutein and MPOD. The independence of these two
295 parameters was checked by assessing the relationship between them (lack of linear Pearson’s
296 correlation). The fact that SNPs were found associated with two independent markers of

297 lutein metabolism by both PLS and univariate analysis reduces the risk of false positive
298 associations. Differences between means obtained in the different genotype groups were
299 analyzed either by ANOVA followed by the *post hoc* Tukey-Kramer test, or by the Student's
300 t-test when only two genotypes were observed. Values of $p < 0.05$ were considered
301 significant. All univariate statistical analyses were performed with Statview software version
302 5.0 (SAS Institute, Cary, NC, U.S.A.).

303 In a third approach we performed an haplotype-based association analysis of data
304 which originated from a cohort of 622 french subjects (see above the characteristic of the
305 subjects). This analysis was performed with the THESIAS software, which is based on the
306 Stochastic-EM algorithm, and which allows to infer haplotypes from genotypic data and to
307 test their associations with phenotypes of interest (43). Analysis were performed after
308 adjustment for the following covariables: gender, age and BMI.

309

310 **Results**

311

312 *Effect of dietary intervention on dietary lutein intake*

313 Dietary lutein intake of the subjects, fell from 1.3 mg/d to 0.03 mg/d after the lutein
314 poor diet, showing a good compliance of the subjects to the recommendations, and increased
315 to 0.4 mg/d during the dietary intervention, which was probably due to the fact that this period
316 occurred during spring where most subjects increase their intake of fruits and vegetables rich
317 in lutein. Note that there was no significant difference between the intake of the two groups at
318 any of these periods..

319

320 *Effect of the dietary intervention on plasma lutein concentrations*

321 Initial plasma lutein concentrations ranged between 110 and 470 nmol/L (290 ± 20
322 nmol/L). This high interindividual variability (CV=32%) did not change when plasma lutein
323 was corrected for plasma cholesterol (CV=36%), nor when it was corrected for
324 lutein+zeaxanthin intake (CV=146%). Surprisingly, the group that took the placebo (placebo
325 group) exhibited a significant ($P < 0.05$) rise in plasma lutein concentration after the dietary
326 intervention (**Figure 1A**). Nevertheless, this increase was much higher in the group that took
327 lutein supplement (+143 %) than in the placebo group (+77 %). Individual plasma lutein
328 responses to the placebo and the lutein supplement are shown in **Figure 1B and 1C**,
329 **respectively**. The interindividual response (change from the initial value) strongly varied,
330 ranging from -100 to 1140 nmol/L. Consequently, the interindividual variability in plasma
331 lutein increased after lutein supplementation (CV= 29% and 56% before and after
332 supplementation, respectively).

333 Because lutein is almost exclusively carried by lipoproteins and because it is the reason
334 why vitamin E (α -tocopherol) is usually corrected for plasma cholesterol, we corrected

335 plasma lutein for plasma cholesterol. This adjustment led to observe a significant increase (P
336 = 0.003) in the plasma lutein/cholesterol ratio after lutein supplementation and no significant
337 increase ($P = 0.067$) of this ratio after supplementation by the placebo (data not shown).

338

339 *Relationships between genetic variants and biomarkers of lutein status*

340 The PLS analysis showed that the best model to explain the variance of both plasma
341 lutein and MPOD was the one with one latent vector only. This model explained 31.4% of the
342 variance of the Y matrix (38.1% for MPOD and 25.1% for plasma lutein). The predictive
343 value of the model was estimated after cross-validation. We obtained an estimated variation
344 of Y explained by the model of 16.1 % (14.6% for MPOD and 18.3% for plasma lutein). The
345 six SNPs selected by the regression model were found in four genes: BCMO1, CD36,
346 ABCG8 and NPC1L1. Coefficients of NPC1L1 were, however, not statistically significant.
347 Furthermore, only the SNP in BCMO1 and two SNPs in CD36 were simultaneously
348 significantly related to the two independent markers of lutein status. Thus association studies
349 described thereafter were performed only with these two genes.

350

351 *Associations between genetic variants and plasma lutein levels*

352 Univariate analysis of plasma lutein showed that this parameter was higher in subjects
353 who carried the CT genotype at BCMO1 rs7501331 than in those who carried the TT
354 genotype (**Figure 2A**), although the difference was not significant. Note that no subject
355 homozygous for the C allele was found in the study cohort. The difference became significant
356 ($P = 0.022$) when plasma lutein was corrected for plasma cholesterol: + 33 % in the subjects
357 with the CT genotype (**Figure 2B**). Finally, no significant difference was observed when
358 plasma lutein was corrected for lutein intake (data not shown).

359 Univariate analysis also showed that subjects with the CC genotype at rs13230419, a
360 CD36 locus) had lower plasma lutein ($P = 0.014$) than subjects with a T allele at this locus
361 (**Figure 3A**). The difference remained significant ($P = 0.044$) when plasma lutein was
362 corrected for plasma cholesterol (**Figure 3B**). Adjustment for lutein intake led to a
363 comparable figure (data not shown).

364 **Tables 3 and 4** show results of haplotypes association analysis between BCMO1 and
365 CD36 haplotypes and plasma lutein/zeaxanthin concentration in a cohort of 622 subjects.
366 There was no significant effect of the BCMO1 haplotypes on plasma lutein, either adjusted or
367 not for plasma cholesterol (Table 3). Conversely, a minor haplotype of CD36 (AGA) had a
368 significant effect on plasma lutein/zeaxanthin concentrations as compared to the most frequent
369 haplotype (AAG). Note that this effect remained significant ($P=0.048$) when plasma
370 lutein/zeaxanthin was adjusted for plasma cholesterol (data not shown).

371

372 *Effect of the dietary intervention on MPOD*

373 MPOD measured before supplementation was not significantly different between the
374 two groups (**Figure 4A**). After the supplementation period, MPOD significantly increased (P
375 < 0.05) in both groups. Again, this increase was higher in the lutein group (+22%) than in the
376 placebo group (+11%) (**Figure 4A**). Interestingly the interindividual variability in initial
377 MPOD (CV=32%, **Figure 4B**) was similar to that observed for plasma lutein and, as observed
378 for plasma lutein, increased when MPOD was corrected for lutein intake (CV=142%).
379 Conversely, in opposite to what was observed for plasma lutein, the interindividual variability
380 in MPOD decreased after supplementation with lutein (CV decreased from 39% to 25%,
381 **Figure 4B**).

382 **Figure 5A** shows that the MPOD response was not related to the plasma lutein
383 response. Furthermore, the initial MPOD was not related to the initial plasma lutein ($r =$

384 0.009, $P = 0.96$), when either corrected or not for plasma cholesterol ($r = 0.20$, $P = 0.30$) (data
385 not shown). Conversely, there was a significant inverse relationship between the MPOD
386 response and the initial MPOD (**Figure 5B**). In other words, the lower the initial MPOD was,
387 the higher the MPOD response to the dietary intervention.

388

389 *Association between genetic variants and MPOD*

390 **Figure 6** shows results of univariate analysis between the SNPs in BCMO1 and CD36
391 and the initial MPOD. Subjects with CT at the BCMO1 SNP had significantly lower initial
392 MPOD values than homozygous TT (**Figure 6A**). Interestingly the CT subjects had a higher
393 MPOD response (delta from initial values) to the lutein supplement than the TT subjects (data
394 not shown). Subjects bearing an A allele at rs1761667 (a CD36 locus) had significantly lower
395 MPOD than homozygous GG (Figure 7B). As observed for BCMO1, the genetic groups with
396 the lower MPOD (the AA and AG groups) had a higher MPOD response to the lutein
397 supplement than the group with the higher MPOD (the GG group) (data not shown).

398

399 Discussion

400

401 Initially, we verified the effect of lutein supplementation on two markers of lutein
402 status, which were plasma lutein and MPOD. The dose of lutein provided by the supplement
403 (10 mg/d) and the duration of the supplementation (6 months) were selected to enable the
404 detection of significant variations in MPOD (44-46). The fact that, for better compliance,
405 lutein was provided as pills instead of foods (spinach, for example) is unlikely to have
406 influenced the results significantly, because it has been shown that lutein bioavailability is not
407 significantly different between spinach and lutein supplements (47, 48). The significant
408 increase of both markers in the lutein supplemented group was expected, because it is in
409 agreement with previous studies (44, 48, 49). The first obvious conclusion is that these
410 increases were due to the lutein present in the supplement. The significant increase of both
411 markers in the placebo (control) group did, however, raise some questions about this
412 conclusion. Nevertheless, it should be remembered that lutein was not only provided by the
413 supplement, but it was also, of necessity, present in the subjects' diets. We thus hypothesized
414 that the increase of the lutein markers in the placebo group was due to an increase in dietary
415 lutein intake. This hypothesis was supported by the fact that a second food diary, kept during
416 the supplementation period, which occurred in spring where subjects increase their intake of
417 lutein-rich fruit and vegetables, showed that all subjects increased their dietary intake of lutein
418 as compared to their initial dietary intake. Given that the food diaries showed that the dietary
419 intake of lutein during the supplementation period was not significantly different between the
420 two groups, this demonstrated that the higher increase of both markers in the lutein
421 supplemented group (+143 % vs. +77 % for plasma lutein and +22 % vs. +11 % for MPOD,
422 in the lutein and placebo groups, respectively) was due to the lutein supplement.

423 MPOD is assumed to be related to the macula lutea concentration of lutein. Because
424 some studies showed a significant relationship between blood lutein and MPOD (36, 50),
425 while other studies did not find this association (49, 51), we aimed to re-assess this
426 relationship. Our results show that MPOD was not related to plasma lutein, whatever the
427 parameter used, such as plasma lutein, plasma lutein corrected for plasma cholesterol or
428 plasma lutein corrected for lutein intake. In fact, this association, when observed, was weak
429 and found only with a large number of subjects (36, 52). Thus, considering the small number
430 of subjects in our study, our result is not surprising. Interestingly, and in agreement with a
431 recent study (53), we found that the MPOD response was inversely and significantly related to
432 the initial MPOD. This shows that the subjects who respond the most to dietary lutein are
433 those who have the lowest initial MPOD. This should be taken into account in future studies
434 of the effect of different parameters on MPOD.

435 The main objective of this study was to check the hypothesis that genetic variants can
436 explain, at least in part, the interindividual variability in blood lutein concentrations and
437 MPOD observed in this study (figures 1 and 5), as well as in previous studies. To that aim, we
438 compared plasma lutein concentrations and MPOD in volunteers bearing different SNPs in
439 genes potentially involved in lutein metabolism. Because we wanted to predict blood lutein
440 concentrations and MPOD (dependent variables) from a large set of SNPs (independent
441 variable), we used PLS regression. As stated in the material and method section this analysis
442 has the advantage of avoiding false positive results that could be obtained in multiple testing
443 and small sample size. This analysis showed that a significant part of the variability (38% for
444 MPOD and 25% for plasma lutein) can be assigned to genetic variants in CD36, BCMO1 and
445 ABCG8. Furthermore, three SNPs were simultaneously and significantly related to plasma
446 lutein and MPOD: one in BCMO1, and two in CD36. Since plasma lutein and MPOD were
447 not related, this double association further supports the involvement of these genes in lutein

448 metabolism. However, in order to reinforce the validity of these associations we decided to
449 perform an haplotype-based association analysis of data which originated from a cohort of
450 622 subjects. This analysis confirmed the association of plasma lutein with CD36 but failed to
451 find a significant association between plasma lutein and BCMO1. This is likely due to an
452 insufficient statistical power because a recent genome wide association study supported a role
453 of BCMO1 in plasma lutein levels (54).

454 The association of plasma lutein concentration with BCMO1 suggests that this gene,
455 which encodes an enzyme responsible for the cleavage of provitamin A carotenoids into
456 retinal (29), is also involved in the metabolism of lutein, which is not a provitamin A
457 carotenoid. This association is probably indirect because zeaxanthin (a xanthophyll which
458 exhibits a chemical structure very close to lutein) was not cleaved *in vitro* by BCMO1 (55),
459 supporting the assumption that BCMO1 is not involved in lutein cleavage. One hypothesis
460 might be that vitamin A status, related to BCMO1 activity, can modulate lutein metabolism
461 by modulating expression of transporters of lutein. This is supported by a publication showing
462 that *all-trans* retinoic acid strongly up-regulate CD36 (56). The association between BCMO1
463 and MPOD was observed for the first time and suggests that BCMO1 activity modulate the
464 concentration of lutein in the retina. The effect of this SNP in BCMO1 on plasma and retina
465 concentration of lutein is probably related to an effect on BCMO1 activity. Indeed this SNP
466 leads to a change in an amino acid (A379V), and this change is functional, as a recombinant
467 267S + 379V double mutant showed a reduced BCMO1 catalytic activity and carriers of the
468 379V genotype had a reduced ability to convert β -carotene (57).

469 The second gene that is apparently involved in lutein metabolism is the scavenger
470 receptor CD36, also known as FAT. This is not very surprising as, SR-BI, a scavenger
471 receptor like CD36, has been involved in the uptake of lutein by both intestinal (13) and
472 retinal cells (28). Because CD36 is expressed in the intestine and in several other tissues, we

473 hypothesize that functional variants in its gene may affect lutein absorption efficiency, or
474 tissue uptake of lutein, or both, leading to lower concentrations of plasma and retina lutein.
475 Unfortunately, there are no functional data available about the effect of the studied variants in
476 CD36 on the activity, or expression, of CD36.

477 The lack of association between the two lutein biomarkers and the SNPs in SCARB1,
478 the gene that encodes SR-BI, was rather unexpected, since this transporter has been involved
479 in lutein absorption (13) and retinal cell uptake (28). Furthermore, two out of the three
480 genotyped SNPs (rs5888 and rs4238001) have been associated with lower SR-BI protein
481 expression and function (58, 59). Thus, the lack of association between these SNPs and
482 plasma lutein and MPOD, together with the fact that CD36 (another scavenger receptor which
483 share many structural characteristics with SR-BI) was associated with plasma and retina
484 lutein, suggests that CD36 is probably more important for lutein uptake by cells than SR-BI
485 and that it can compensate for lower expression/function of SR-BI.

486 The fact that two genes were associated with the studied biomarkers of lutein status
487 raises questions about interactions between these genes. Unfortunately, the number of subjects
488 enrolled in this study was too low to detect a significant interaction between BCMO1 and
489 CD36 (two factors ANOVA).

490

491 In summary, our results suggest that genetic variants in BCMO1 and CD36 modulate
492 plasma and retina lutein concentrations. This observation may have consequences with regard
493 to the recommended dietary intake of lutein in groups of subjects bearing unfavourable
494 genetic variants in these genes.

495

496 **References**

- 497 1. Evans JR. Risk factors for age-related macular degeneration. *Prog Retin Eye Res.* 2001 Mar;20(2):227-53.
498 2. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in
499 the eye. *Annu Rev Nutr.* 2003;23:171-201.
500 3. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys.*
501 2001;385(1):28-40.
502 4. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM. Nutritional manipulation of primate
503 retinas, III: Effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free
504 monkeys. *Invest Ophthalmol Vis Sci.* 2005 Feb;46(2):692-702.
505 5. Tyssandier V, Reboul E, Dumas JF, Bouteloup-Demange C, Armand M, Marcand J, et al. Processing of
506 vegetable-borne carotenoids in the human stomach and duodenum. *Am J Physiol Gastrointest Liver*
507 *Physiol.* 2003 Jun;284(6):G913-23.
508 6. Reboul E, Richelle M, Perrot E, Desmoulins-Malezet C, Pirisi V, Borel P. Bioaccessibility of Carotenoids
509 and Vitamin E from Their Main Dietary Sources. *J Agric Food Chem.* 2006 Nov 15;54(23):8749-55.
510 7. Borel P, Grolier P, Armand M, Partier A, Lafont H, Lairon D, et al. Carotenoids in biological emulsions:
511 solubility, surface-to-core distribution, and release from lipid droplets. *J Lipid Res.* 1996;37(2):250-61.
512 8. Tyssandier V, Lyan B, Borel P. Main factors governing the transfer of carotenoids from emulsion lipid
513 droplets to micelles. *Biochim Biophys Acta.* 2001;1533(3):285-92.
514 9. Borel P. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble
515 vitamins, carotenoids and phytosterols). *Clin Chem Lab Med.* 2003 Aug;41(8):979-94.
516 10. Hollander D, Muralidhara KS. Vitamin A1 intestinal absorption in vivo: influence of luminal factors on
517 transport. *Am J Physiol Gastrointest Liver Physiol.* 1977;232:E471-E7.
518 11. Hollander D. Intestinal absorption of vitamin A, E, D, and K. *J Lab Clin Med.* 1981;97:449-62.
519 12. Hollander D, Ruble PE. beta-carotene intestinal absorption: bile, fatty acid, pH, and flow rate effects on
520 transport. *Am J Physiol.* 1978;235(6):E686-91.
521 13. Reboul E, Abou L, Mikail C, Ghiringhelli O, Andre M, Portugal H, et al. Lutein transport by Caco-2 TC-7
522 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). *Biochem*
523 *J.* 2005 Apr 15;387(Pt 2):455-61.
524 14. van Bennekum A, Werder M, Thuahnai ST, Han CH, Duong P, Williams DL, et al. Class B Scavenger
525 Receptor-Mediated Intestinal Absorption of Dietary beta-Carotene and Cholesterol. *Biochemistry (Mosc).*
526 2005 Mar 22;44(11):4517-25.
527 15. During A, Dawson HD, Harrison EH. Carotenoid Transport Is Decreased and Expression of the Lipid
528 Transporters SR-BI, NPC1L1, and ABCA1 Is Downregulated in Caco-2 Cells Treated with Ezetimibe. *J*
529 *Nutr.* 2005 Oct;135(10):2305-12.
530 16. During A, Harrison EH. Mechanisms of provitamin A (carotenoid) and vitamin A (retinol) transport into
531 and out of intestinal Caco-2 cells. *J Lipid Res.* 2007 Oct;48(10):2283-94.
532 17. Moussa M, Landrier JF, Reboul E, Ghiringhelli O, Comera C, Collet X, et al. Lycopene absorption in
533 human intestinal cells and in mice involves scavenger receptor class B type I but not niemann-pick C1-like
534 1. *J Nutr.* 2008 Aug;138(8):1432-6.
535 18. Davis HR, Jr., Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, et al. Niemann-Pick C1 Like 1 (NPC1L1)
536 is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol
537 homeostasis. *J Biol Chem.* 2004 Aug 6;279(32):33586-92.
538 19. Narushima K, Takada T, Yamanashi Y, Suzuki H. Niemann-pick C1-like 1 mediates alpha-tocopherol
539 transport. *Mol Pharmacol.* 2008 Jul;74(1):42-9.
540 20. Reboul E, Klein A, Bietrix F, Gleize B, Malezet-Desmoulins C, Schneider M, et al. Scavenger receptor
541 class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. *J Biol Chem.* 2006 Feb
542 24;281(8):4739-45.
543 21. Lee MH, Lu K, Patel SB. Genetic basis of sitosterolemia. *Curr Opin Lipidol.* 2001;12(2):141-9.
544 22. Oram JF. Tangier disease and ABCA1. *Biochim Biophys Acta.* 2000 Dec 15;1529(1-3):321-30.
545 23. Oram JF, Vaughan AM, Stocker R. ATP-binding cassette transporter A1 mediates cellular secretion of
546 alpha-tocopherol. *J Biol Chem.* 2001 Oct 26;276(43):39898-902.
547 24. Reboul E, Tromprier D, Moussa M, Klein A, Landrier JF, Chimini G, et al. ATP-binding cassette transporter
548 A1 is significantly involved in the intestinal absorption of alpha- and gamma-tocopherol but not in that of
549 retinyl palmitate in mice. *Am J Clin Nutr.* 2009 Jan;89(1):177-84.
550 25. Tyssandier V, Choubert G, Grolier P, Borel P. Carotenoids, mostly the xanthophylls, exchange between
551 plasma lipoproteins. *Int J Vitam Nutr Res.* 2002;72(5):300-8.
552 26. Borel P, Moussa M, Reboul E, Lyan B, Defoort C, Vincent-Baudry S, et al. Human plasma levels of
553 vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid
554 metabolism. *J Nutr.* 2007 Dec;137(12):2653-9.

- 555 27. Borel P, Moussa M, Reboul E, Lyan B, Defoort C, Vincent-Baudry S, et al. Human fasting plasma
556 concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III,
557 cholesteryl ester transfer protein, hepatic lipase, intestinal fatty acid binding protein and microsomal
558 triacylglycerol transfer protein. *Br J Nutr.* 2008 Jul 29;118:1-8.
- 559 28. During A, Doraiswamy S, Harrison EH. Xanthophylls are preferentially taken up compared with beta-
560 carotene by retinal cells via a SRBI-dependent mechanism. *J Lipid Res.* 2008 Aug;49(8):1715-24.
- 561 29. von Lintig J, Vogt K. Vitamin A formation in animals: molecular identification and functional
562 characterization of carotene cleaving enzymes. *J Nutr.* 2004 Jan;134(1):251S-6S.
- 563 30. Vage DI, Boman IA. A nonsense mutation in the beta-carotene oxygenase 2 (BCO2) gene is tightly
564 associated with accumulation of carotenoids in adipose tissue in sheep (*Ovis aries*). *BMC Genet.* 11:10.
- 565 31. Hercberg S, Deheeger M, Preziosi P. SU.VI.MAX: Portions alimentaires. Manuel photos pour l'estimation
566 des quantités. 1994;Su.Vi.Max.-Candia-Polytechnica.
- 567 32. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E. The development and
568 application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. *J Am Diet*
569 *Assoc.* 1993;93(3):318-23.
- 570 33. Cardinault N, Tyssandier V, Grolier P, Winklhofer-Roob BM, Ribalta J, Bouteloup-Demange C, et al.
571 Comparison of the postprandial chylomicron carotenoid responses in young and older subjects. *Eur J Nutr.*
572 2003 Dec;42(6):315-23.
- 573 34. Tyssandier V, Feillet-Coudray C, Caris-Veyrat C, Guillard JC, Coudray C, Bureau S, et al. Effect of tomato
574 product consumption on the plasma status of antioxidant microconstituents and on the plasma total
575 antioxidant capacity in healthy subjects. *J Am Coll Nutr.* 2004 Apr;23(2):148-56.
- 576 35. Caris-Veyrat C, Amiot MJ, Tyssandier V, Grasselly D, Buret M, Mikolajczak M, et al. Influence of organic
577 versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and
578 derived purees; consequences on antioxidant plasma status in humans. *J Agric Food Chem.* 2004 Oct
579 20;52(21):6503-9.
- 580 36. Curran-Celentano J, Hammond BR, Jr., Ciulla TA, Cooper DA, Pratt LM, Danis RB. Relation between
581 dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a
582 Midwest population. *Am J Clin Nutr.* 2001;74(6):796-802.
- 583 37. De la Vega FM, Lazaruk KD, Rhodes MD, Wenz MH. Assessment of two flexible and compatible SNP
584 genotyping platforms: TaqMan SNP Genotyping Assays and the SNPlex Genotyping System. *Mutat Res.*
585 2005 Jun 3;573(1-2):111-35.
- 586 38. Tobler AR, Short S, Andersen MR, Paner TM, Briggs JC, Lambert SM, et al. The SNPlex genotyping
587 system: a flexible and scalable platform for SNP genotyping. *J Biomol Tech.* 2005 Dec;16(4):398-406.
- 588 39. Zagers NPA, van Norren D. Absorption of the eye lens and macular pigment derived from the reflectance of
589 cone photoreceptors. *J Opt Soc Am.* 2004;21:2257-68
- 590 40. van de Kraats J, van Norren D. Directional and nondirectional spectral reflection from the human fovea *J*
591 *Biomed Opt.* 2008;13(2):024010.
- 592 41. Lyan B, Azais-Braesco V, Cardinault N, Tyssandier V, Borel P, Alexandre-Gouabau MC, et al. Simple
593 method for clinical determination of 13 carotenoids in human plasma using an isocratic high-performance
594 liquid chromatographic method. *J Chromatogr B Biomed Appl.* 2001;751(2):297-303.
- 595 42. Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, et al. The SU.VI.MAX Study: a
596 randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. *Arch Intern*
597 *Med.* 2004 Nov 22;164(21):2335-42.
- 598 43. Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL. A new algorithm for haplotype-based association
599 analysis: the Stochastic-EM algorithm. *Ann Hum Genet.* 2004 Mar;68(Pt 2):165-77.
- 600 44. Johnson EJ, Chung HY, Caldarella SM, Snodderly DM. The influence of supplemental lutein and
601 docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. *Am J Clin Nutr.* 2008
602 May;87(5):1521-9.
- 603 45. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, et al. Double-masked, placebo-controlled,
604 randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related
605 macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry.*
606 2004 Apr;75(4):216-30.
- 607 46. Bone RA, Landrum JT, Guerra LH, Ruiz CA. Lutein and Zeaxanthin Dietary Supplements Raise Macular
608 Pigment Density and Serum Concentrations of these Carotenoids in Humans. *J Nutr.* 2003;133(4):992-8.
- 609 47. Riso P, Brusamolino A, Ciappellano S, Porrini M. Comparison of lutein bioavailability from vegetables and
610 supplement. *Int J Vitam Nutr Res.* 2003 May;73(3):201-5.
- 611 48. Chung HY, Rasmussen HM, Johnson EJ. Lutein bioavailability is higher from lutein-enriched eggs than
612 from supplements and spinach in men. *J Nutr.* 2004 Aug;134(8):1887-93.

- 613 49. Cardinault N, Gorrard JM, Tyssandier V, Grolier P, Rock E, Borel P. Short-term supplementation with
614 lutein affects biomarkers of lutein status similarly in young and elderly subjects. *Exp Gerontol*. 2003
615 May;38(5):573-82.
- 616 50. Broekmans WM, Berendschot TT, Klopping-Ketelaars IA, de Vries AJ, Goldbohm RA, Tijburg LB, et al.
617 Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum
618 concentrations of zeaxanthin. *Am J Clin Nutr*. 2002;76(3):595-603.
- 619 51. Johnson EJ, Hammond BR, Yeum KJ, Qin J, Wang XD, Castaneda C, et al. Relation among serum and
620 tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr*.
621 2000;71(6):1555-62.
- 622 52. Bone RA, Landrum JT, Dixon Z, Chen Y, Llerena CM. Lutein and zeaxanthin in the eyes, serum and diet of
623 human subjects. *Exp Eye Res*. 2000 Sep;71(3):239-45.
- 624 53. Richer S, Devenport J, Lang JC. LAST II: Differential temporal responses of macular pigment optical
625 density in patients with atrophic age-related macular degeneration to dietary supplementation with
626 xanthophylls. *Optometry*. 2007 May;78(5):213-9.
- 627 54. Ferrucci L, Perry JR, Matteini A, Perola M, Tanaka T, Silander K, et al. Common Variation in the beta-
628 Carotene 15,15'-Monooxygenase 1 Gene Affects Circulating Levels of Carotenoids: A Genome-Wide
629 Association Study. *Am J Hum Genet*. 2009 Jan 28.
- 630 55. Grolier P, Duszka C, Borel P, Alexandre-Gouabau MC, Azais-Braesco V. In vitro and in vivo inhibition of
631 beta-carotene dioxygenase activity by canthaxanthin in rat intestine. *Arch Biochem Biophys*.
632 1997;348(2):233-8.
- 633 56. Langmann T, Liebisch G, Moehle C, Schifferer R, Dayoub R, Heiduczek S, et al. Gene expression profiling
634 identifies retinoids as potent inducers of macrophage lipid efflux. *Biochim Biophys Acta*. 2005;1740:155-
635 61.
- 636 57. Leung WC, Hessel S, Meplan C, Flint J, Oberhauser V, Tourniaire F, et al. Two common single nucleotide
637 polymorphisms in the gene encoding {beta}-carotene 15,15'-monooxygenase alter {beta}-carotene
638 metabolism in female volunteers. *FASEB J*. 2008 Dec 22.
- 639 58. West M, Greason E, Kolmakova A, Jahangiri A, Asztalos B, Pollin TI, et al. Scavenger receptor class B
640 type I protein as an independent predictor of high-density lipoprotein cholesterol levels in subjects with
641 hyperalphalipoproteinemia. *J Clin Endocrinol Metab*. 2009 Apr;94(4):1451-7.
- 642 59. Constantineau J, Greason E, West M, Filbin M, Kieft JS, Carletti MZ, et al. A synonymous variant in
643 scavenger receptor, class B, type I gene is associated with lower SR-BI protein expression and function.
644 *Atherosclerosis*. 2010;210:177-82.
- 645
646

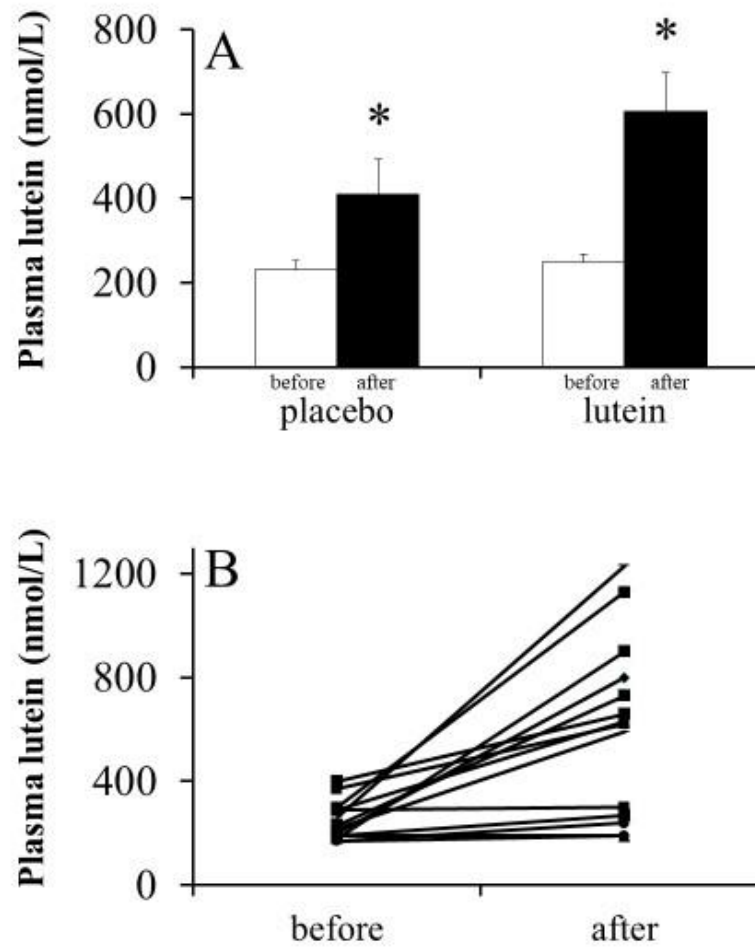


Figure 1

648

649

650 **Figure 1:** (A) Plasma lutein concentrations, before and after supplementation, in both the group that took the

651 placebo and the group that took the lutein supplement. White bars: values measured before supplementation.

652 Black bars: values measured after supplementation. Means \pm SEM of 15 subjects in placebo group and 14653 subjects in lutein group. An asterisk indicates a significant difference ($P < 0.05$) between values measured before

654 and after the supplementation period in each group (paired t test). (B) Individual plasma lutein concentrations,

655 before and after supplementation, in the placebo group. (C) Individual plasma lutein concentrations, before and

656 after supplementation, in the lutein supplemented group.

657

658

659

660

661

662

663

664

665

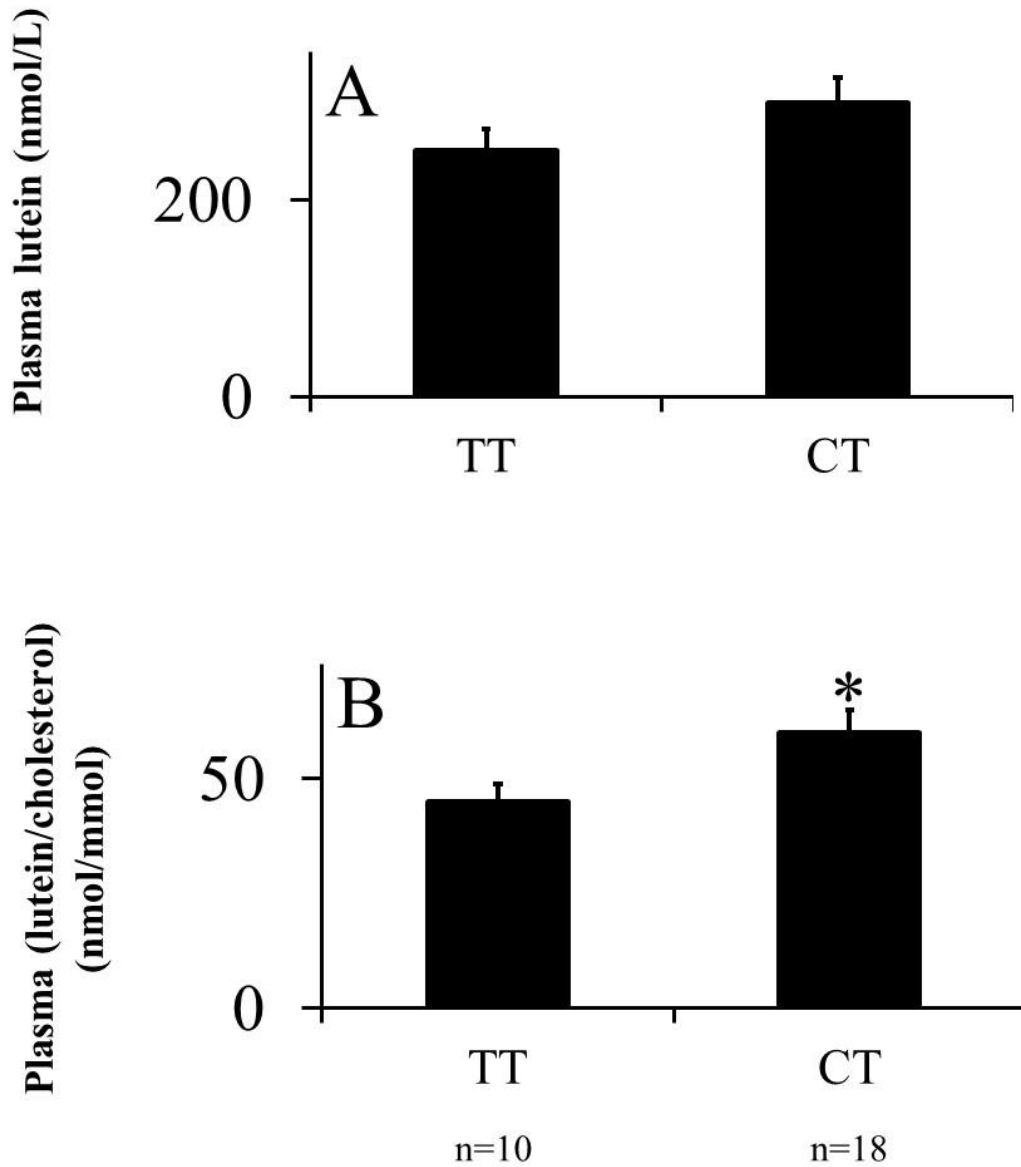
666

667

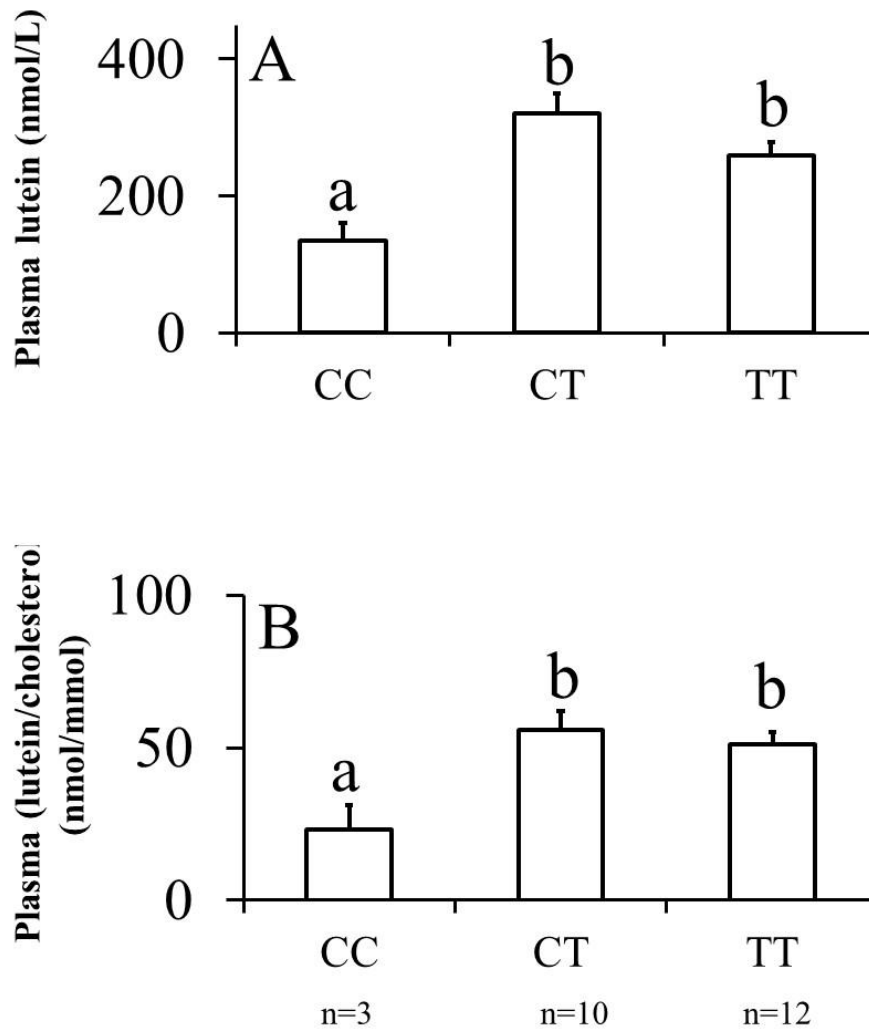
668

669

670



671
672 **Figure 2:** Baseline plasma lutein concentrations for each BCMO1 rs7501331 genotype. (A) uncorrected values.
673 (B) values corrected for plasma total cholesterol. Data are means \pm SEM of 28 subjects. n is the number of
674 subject in each genotype group. An asterisk indicates a significant difference ($P < 0.05$) between genotype
675 groups (Student's t test).
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691



692 **Figure 3:** Baseline plasma lutein concentrations for each CD36 rs13230419 genotype. (A) uncorrected values.
 693 (B) Values corrected for plasma total cholesterol. Data are means \pm SEM of 25 subjects. n is the number of
 694 subject in each genotype group. In each figure, different letters indicate significant differences ($P < 0.05$)
 695 between genotype groups (ANOVA).
 696
 697
 698
 699
 700
 701
 702
 703
 704
 705
 706
 707
 708
 709
 710
 711
 712
 713
 714

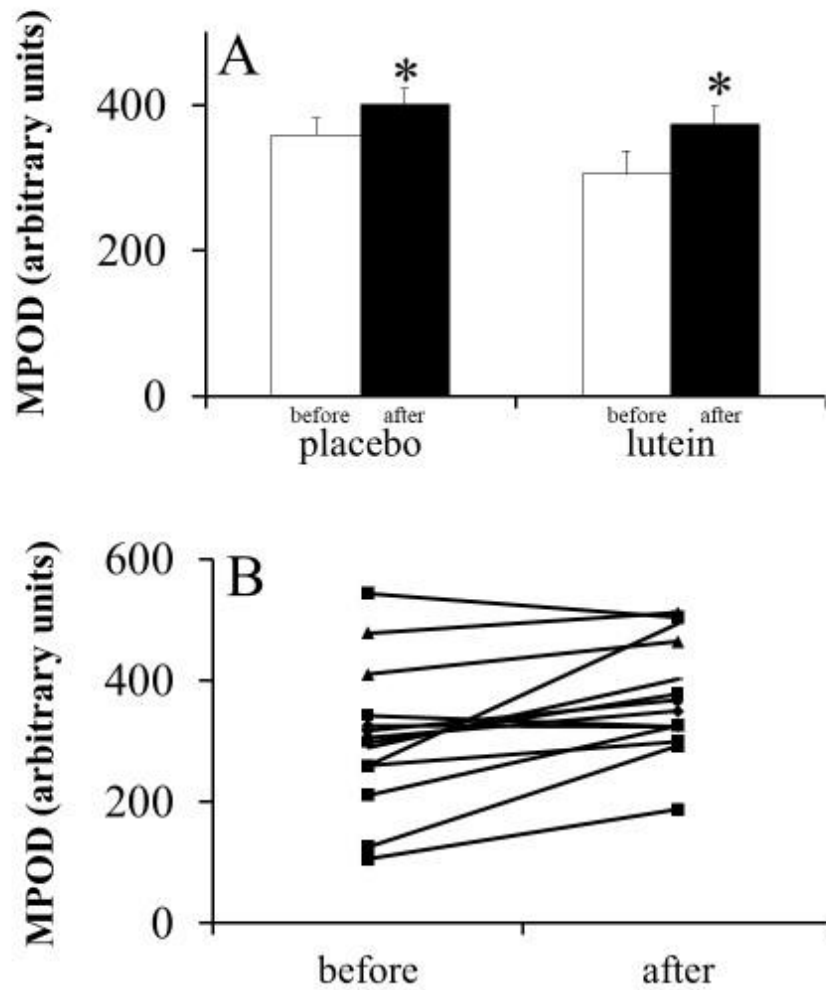
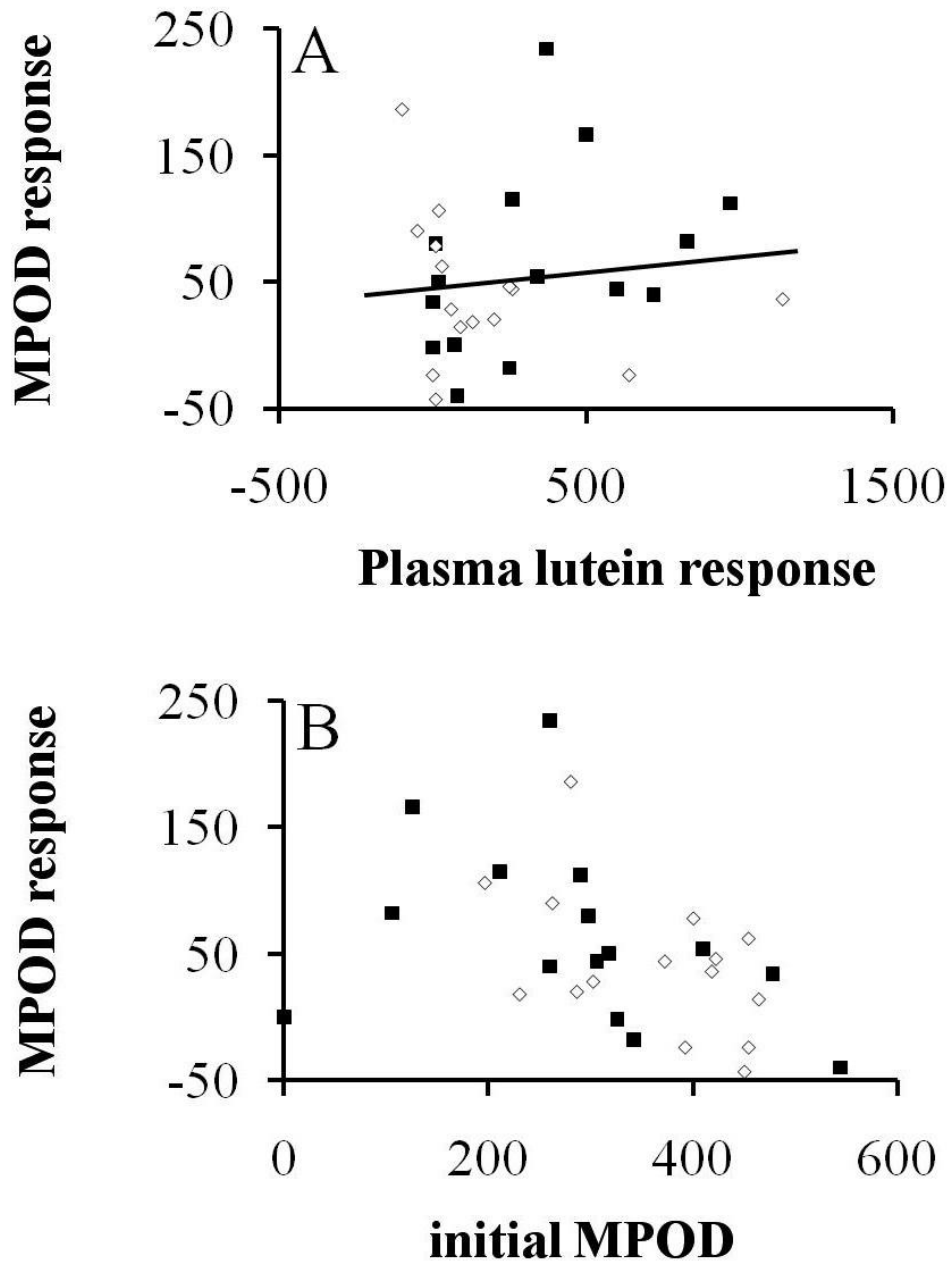


Figure 4

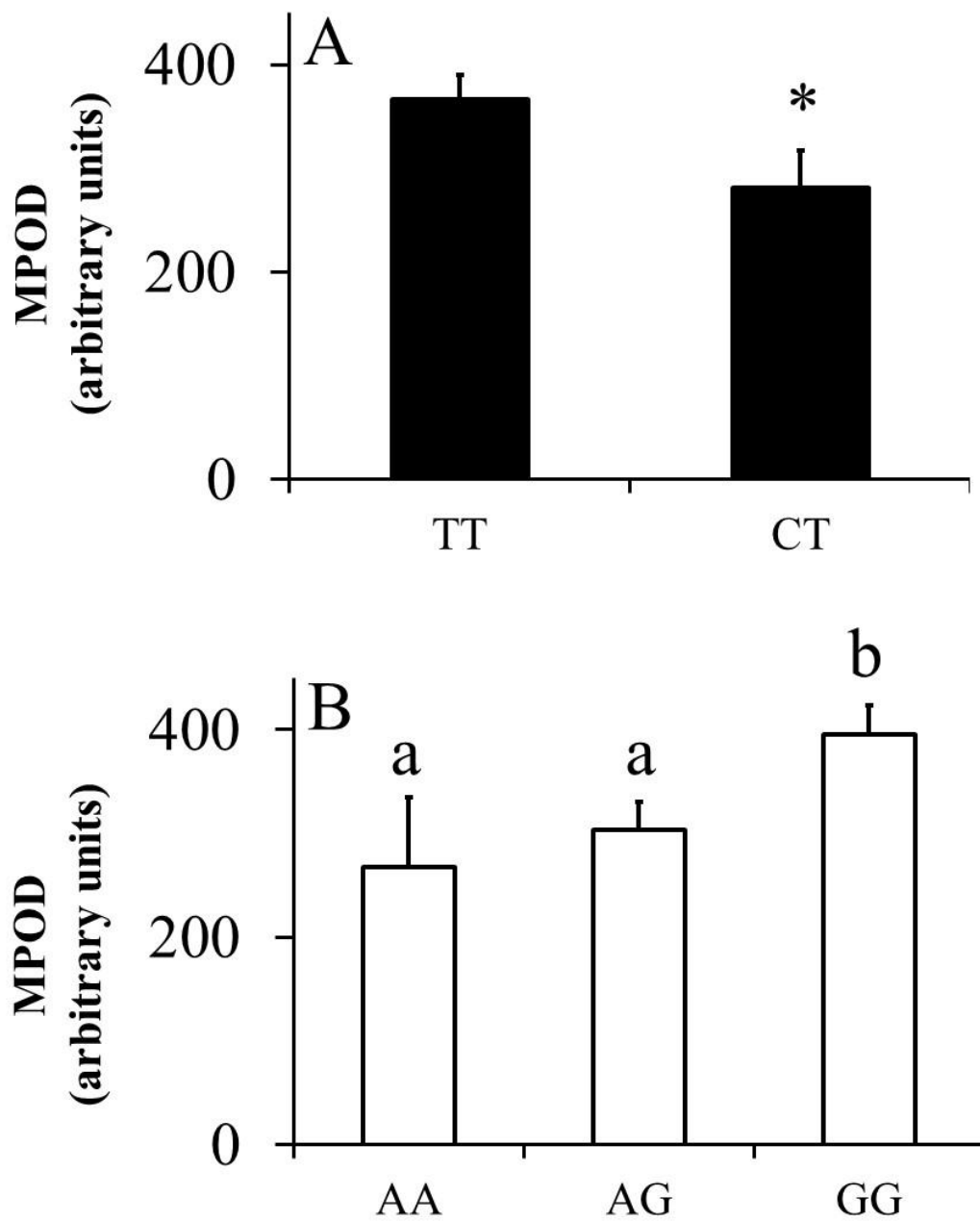
Figure 4: (A) MPOD (macular pigment optical density), before and after supplementation, in the group that took the placebo and the group that took the lutein supplement. White bars: values measured before supplementation (before). Black bars: values measured after supplementation (after). Means \pm SEM of 15 subjects in placebo group and 14 subjects in lutein group. An asterisk indicates a significant difference ($P < 0.05$) between values measured before and after the supplementation period in each group (paired t test). (B) Individual MPOD before and after supplementation in the lutein supplemented group (n=14).

715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735



736
 737
 738
 739
 740
 741
 742
 743
 744
 745
 746
 747
 748

Figure 5: (A) MPOD response as a function of plasma lutein response. (B) MPOD response as a function of initial MPOD. “Response” indicates the differences between the values measured after the dietary intervention and the values measured before the intervention ($n = 29$). White dots: subjects in the placebo group, black dots: subjects in the lutein supplemented group.



749
750
751
752
753
754
755

Figure 6: MPOD for each BCMO1 and CD36 genotype. (A) BCMO1 rs7501331 genotypes. An asterisk indicates a significant difference ($P < 0.05$) between genotype groups (Student's t test). (B) CD36 rs1761667 genotypes. Different letters indicate significant differences ($P < 0.05$) between genotype groups (ANOVA). n is the number of subject in each genotype group.

756 **Tables**

757

758 **Table 1** Characteristics and nutrient intake of the 29 male subjects enrolled in the clinical study¹

Item	Mean ± SD
Age, <i>y</i>	49.7 ± 0.8
Height, <i>cm</i>	174.2 ± 1.2
Weight, <i>kg</i>	71.1 ± 1.3
Plasma analytes	
Glucose, <i>mmol/L</i>	4.88 ± 0.09
Triglycerides, <i>mmol/L</i>	0.93 ± 0.07
Total cholesterol, <i>mmol/L</i>	4.91 ± 0.14
HDL cholesterol, <i>mmol/L</i>	1.63 ± 0.08
LDL cholesterol, <i>mmol/L</i>	2.99 ± 0.13
Nutrient intake ¹	
Total Energy, <i>kJ/d</i>	2406 ± 103
Alcohol, <i>g/d</i>	9.0 ± 2.2
Carbohydrate, <i>g/d</i>	279.1 ± 14.2
Protein, <i>g/d</i>	94.2 ± 4.4
Fat, <i>g/d</i>	98.0 ± 4.9
Lutein+zeaxanthin <i>mg/d</i>	1.3 ± 0.4

759

760 ¹Nutrient intake of the subjects as evaluated, at the beginning of the study (i.e. before the lutein-poor diet), by a

761 3-day food diary.

762

763 **Table 2** Characteristics of the studied SNPs

764

Gene	dbSNP ID	SNP ID	Main allele	Minor allele	MAF (%) ¹	Intron (I)/Exon (E)/N(Near the gene)	Possible effect on protein activity/expression
ABCA1	rs2230808	hCV2741104	G	A	24.3	E	NS ²
ABCA1	rs2066718	hCV11720789	G	A	3.5	E	NS
ABCA1	rs2230806	hCV2741051	G	A	28.8	E	NS
ABCA1	rs2230805	hCV2741050	G	A	27.4	E	silent
ABCG5	rs6720173	hCV29001998	G	C	15.2	E	NS
ABCG8	rs4148211	hCV29535502	A	G	35.0	E	NS
ABCG8	rs4148217	hCV375061	C	A	20.2	E	NS
ABCG8	rs6544718	hCV25642779	C	T	20.5	E	NS
BCDO2	rs7123686	hCV22274763	A	C	33.6	I	-
BCMO1	rs12934922	hCV25745282	A	T	45.7	E	NS
BCMO1	rs7501331	hCV ³	T	C	23.6	E	NS
CD36	rs1527483	hCV8315330	C	T	8.0	I	-
CD36	rs1761667	hCV8314499	A	G	45.1	N	?
CD36	rs13230419	hCV1803768	T	C	44.0	N	?
CD36	rs7755	hCV8315318	G	A	43.9	N	?
CD36	rs1984112	hCV12093946	A	G	35.8	N	?
NPC1L1	rs217434	hCV2292875	A	G	18.2	E	silent
NPC1L1	rs217428	hCV29736499	T	G	24.1	I	-
NPC1L1	rs17655652	hCV2625150	T	C	31.8	N	?
SCARB1	rs5888	hCV7497008	C	T	47.2	E	silent
SCARB1	rs4238001	hCV26062113	C	T	13.0	E	NS
SCARB1	intron 5 ³	hCV25608758	C	T	10.0	I	-

765

766 ¹MAF: minor allele frequency as observed in a cohort of 2994 french subjects which was a subset of the french767 SUVIMAX cohort (42). ²NS: non synonymous SNP (missense), indicating a change of amino acid. ³not

768 referenced in SNP ID or dbSNP. ?: effect not known, although it may have a possible effect on gene expression.

769 SNPs were genotyped with the SNPlex assay except hCV26062113 and hCV25608758, which were genotyped

770 by Taqman (see Materials and methods).

771

772

773

774

775

776

777

778 **Table 3** BCMO1 haplotypes effects on plasma lutein/zeaxanthin

779

780 Haplotypes

rs12934922	rs7501331	Frequencies ¹ (%)	Haplotype effect ²
A	C	34.1	0.571
A	T	21.1	0.887
T	C	42.7	-
T	T	2.1	0.368

781

782 Plasma lutein/zeaxanthin and BCMO1 SNPs were assessed in 622 french subjects (281 males, 341 females).

783 ¹Haplotype frequencies were estimated using Thesias software (43). The most frequent haplotype (TC) is the

784 reference haplotype. ²Haplotype effect on plasma lutein/zeaxanthin by comparison to the most frequent

785 haplotype (p value calculated by Student t-test).

786

787

788 **Table 4** CD36 haplotypes effects on plasma lutein/zeaxanthin

789

rs1984112	rs1761667	rs7755	Haplotype Frequencies ¹ (%)	Haplotype effect ²
A	A	A	10.2	0.992
A	A	G	45.2	-
A	G	A	8.4	0.018
A	G	G	1.9	0.567
G	A	G	0.1	- ³
G	G	A	26.2	0.908
G	G	G	7.9	0.074

790

791 Plasma lutein/zeaxanthin and CD36 SNPs were assessed in 622 french subjects (281 males, 341 females).

792 ¹Haplotype frequencies were estimated using Thesias software (43). The most frequent haplotype (AAG) is the793 reference haplotype. ²Haplotype effect on plasma lutein/zeaxanthin by comparison to the most frequent794 haplotype (p value calculated by Student t-test). ³could not be calculated because of the too low frequency of this

795 haplotype.

796

797 **Acknowledgement**

798

799 *Financial support:* Work on the cohort of 29 subjects was funded by Pileje. Pileje participated

800 in the design of the study and approval of the manuscript. Work on the subset cohort of

801 SUVIMAX was granted by ANR (n°ANR-05-PNRA-010), DGS (Ministry of Health) and

802 supported by Médéric, Ipsen, MGEN, SODEXHO and Pierre Fabre. None of these

803 organisations or companies participated in the design or conducted of this research.

804

805 *Authors with financial/conflict of interest:* Stéphanie Baudry, Sophie Drouault-Holowacz,

806 Severine Bieuvelet.

807

808

809