



**HAL**  
open science

## Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP

Patrick Borel, Myriam Moussa, Emmanuelle Reboul, Bernard Lyan, Catherine Defoort, Stéphanie Vincent-Baudry, Mathieu Maillot, Marguerite Gastaldi, Michel Darmon, Henri Portugal, et al.

### ► To cite this version:

Patrick Borel, Myriam Moussa, Emmanuelle Reboul, Bernard Lyan, Catherine Defoort, et al.. Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. *British Journal of Nutrition*, 2009, 101 (5), pp.680-687. 10.1017/S0007114508030754 . hal-02658792

**HAL Id: hal-02658792**

**<https://hal.inrae.fr/hal-02658792v1>**

Submitted on 30 May 2020

**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 Human fasting plasma concentrations of vitamin E and carotenoids,  
2 and their association with genetic variants in apo C-III, CETP, hepatic  
3 lipase, intestinal fatty acid binding protein and MTP

4  
5 Patrick Borel<sup>1,2,3\*</sup>, Myriam Moussa<sup>1,2,3</sup>, Emmanuelle Reboul<sup>1,2,3</sup>, Bernard Lyan<sup>4</sup>, Catherine  
6 Defoort<sup>1,2,3</sup>, Stéphanie Vincent-Baudry<sup>1,2,3</sup>, Matthieu Maillot<sup>1,2,3</sup>, Marguerite Gastaldi<sup>1,2,3</sup>,  
7 Michel Darmon<sup>1,2,3</sup>, Henri Portugal<sup>1,2,3</sup>, Denis Lairon<sup>1,2,3</sup> and Richard Planells<sup>1,2,3</sup>

8  
9 <sup>1</sup> INRA, UMR1260 «Nutriments Lipidiques et Prévention des Maladies Métaboliques», F-  
10 13385 Marseille, France

11 <sup>2</sup> INSERM, U476, F-13385 Marseille, France

12 <sup>3</sup> Univ Aix-Marseille 1, Univ Aix-Marseille 2, Faculté de Médecine, IPHM-IFR 125, F-  
13 13385 Marseille, France

14 <sup>4</sup> INRA, UMR1019 « Nutrition Humaine », Saint-Genes-Champanelle, F-63122 France.

15  
16 Author for correspondence and proofs:

17 Patrick BOREL, Ph.D.

18 UMR 1260 INRA / 476 INSERM / Université Aix-Marseille I et II “Nutriments Lipidiques et  
19 Prévention des Maladies Métaboliques”

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

21 Phone number: (+33) 4 91 29 41 11; FAX number: (+33) 4 91 78 21 01

22 E-mail: [patrick.borel@univmed.fr](mailto:patrick.borel@univmed.fr)

23  
24 Short title: Association between SNPs and plasma vitamin E and carotenoids.

25  
26 Key words: carotene, I-FABP, lycopene, polymorphism, SNP, alpha-tocopherol, gamma-  
27 tocopherol.

28 Abstract

29

30 Plasma concentrations of vitamin E and carotenoids are governed by several factors,  
31 including genetic factors. Single nucleotide polymorphisms (SNPs) in some genes involved in  
32 lipid metabolism have recently been associated with fasting plasma concentrations of these  
33 fat-soluble micronutrients. To further investigate the role of genetic factors that modulate the  
34 plasma concentrations of these micronutrients, we assessed whether SNPs in five candidate  
35 genes (*apo C-III*, *CETP*, *hepatic lipase*, *I-FABP* and *MTP*) were associated with the plasma  
36 concentrations of these micronutrients. Fasting plasma vitamin E and carotenoid  
37 concentrations were measured in 128 French Caucasian subjects (48 males and 80 females).  
38 Candidate SNPs were genotyped by PCR amplification followed by RFLP. Plasma gamma-  
39 tocopherol, alpha-carotene and beta-carotene concentrations were significantly different ( $P <$   
40  $0.05$ ) in subjects who carried different SNP variants in hepatic lipase. Plasma alpha-  
41 tocopherol concentrations were significantly different in subjects who had different SNP  
42 variants in apo C-III and CETP. Plasma lycopene concentrations were significantly different  
43 ( $P < 0.05$ ) in women who had different SNP variants in I-FABP. Finally, there was no effect  
44 of SNP variants in MTP upon the plasma concentrations of these micronutrients. Most of the  
45 observed differences remained significant after the plasma micronutrients were adjusted for  
46 plasma triglycerides and cholesterol. These results suggest that apo C-III, CETP and hepatic  
47 lipase play a role in determining the plasma concentrations of tocopherols while hepatic  
48 lipase and I-FABP may modulate plasma concentrations of carotenoids.

49

## 49 Introduction

50

51 Vitamin E and carotenoids are the main fat-soluble antioxidants found in the human  
52 diet. Although eight forms of vitamin E and more than six hundred carotenoids have been  
53 discovered in nature, two forms of vitamin E ( $\alpha$ - and  $\gamma$ -tocopherol) and six carotenoids ( $\alpha$  and  
54  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, lycopene and zeaxanthin) are present in significant  
55 amounts in human blood and tissues. Research is currently being carried out in order to  
56 understand how consumption of these molecules may be related to the prevention of several  
57 diseases, such as cancer <sup>(1)</sup>, cardio-vascular disease <sup>(2)</sup> and eye disease <sup>(3)</sup>. Since the  
58 absorption of these micronutrients is not very efficient and is highly variable, the mechanisms  
59 involved in the intestinal uptake of these molecules is an active area of research <sup>(4)</sup>. Recent  
60 studies have established that the intestinal absorption of these compounds involves the class B  
61 type I scavenger receptor (SR-BI) <sup>(5-9)</sup>. After uptake, it is assumed that these molecules are  
62 incorporated into chylomicrons and then secreted into the lymph. The protein(s) involved in  
63 the intracellular transport of these hydrophobic molecules in the aqueous environment of the  
64 enterocyte has(ve) not yet been identified, although the cytosolic fatty acid binding proteins  
65 (I-FABP and L-FABP) are likely candidates. Part of the mechanism involved in  $\alpha$ -tocopherol  
66 incorporation in chylomicrons has been recently elucidated. The microsomal triglyceride  
67 transfer protein (MTP) results in a significant decrease in the secretion of vitamin E with  
68 triglyceride-rich lipoproteins and underscore the importance of the chylomicron pathway in  
69 net vitamin E secretion <sup>(10)</sup>. Unfortunately, its role in carotenoid incorporation into  
70 chylomicrons has not yet been studied. Chylomicron vitamin E and carotenoids are  
71 transported to the liver, where they are either stored or distributed to body tissues through  
72 plasma lipoproteins <sup>(11,12)</sup>. Therefore, proteins involved in lipoprotein metabolism are likely  
73 to be involved in the metabolism of these micronutrients. This hypothesis is supported by the  
74 observations that the exchange of vitamin E between lipoproteins <sup>(13,14)</sup> is mediated by  
75 phospholipid transfer protein <sup>(15,16)</sup>, and SR-BI is involved in the transfer of  $\alpha$ -tocopherol  
76 from HDL to tissues <sup>(17)</sup>. It is now clear that proteins involved in lipid metabolism are also  
77 involved in the absorption, intracellular trafficking and plasma transport of vitamin E and  
78 carotenoids. Since single nucleotide polymorphisms (SNPs) in some genes encoding these  
79 proteins have been found to be related to the fasting plasma concentrations of these  
80 micronutrients <sup>(18-20)</sup>, we designed this study to assess whether SNPs in other candidate genes  
81 involved in lipid transport and metabolism are associated with the fasting plasma

82 concentrations of these micronutrients. The presence of such associations would suggest that  
83 these genes and their products play either a direct or indirect role in the metabolism of these  
84 micronutrients. The first gene studied encodes apoprotein C-III, which inhibits triglyceride  
85 removal from the plasma <sup>(21)</sup>. The second gene encodes hepatic lipase (HL), which along with  
86 lipoprotein lipase, is responsible for the lipolysis of lipoprotein triglycerides in the circulation  
87 <sup>(22)</sup>. The third gene encodes I-FABP, which is involved in the intracellular transport of fatty  
88 acids in the small intestine <sup>(23)</sup>. The fourth gene encodes a protein in charge of the  
89 incorporation of triglycerides in chylomicrons, microsomal triglyceride transfer protein  
90 (MTP) <sup>(24)</sup>. The fifth gene encodes a protein, cholesterol ester transfer protein (CETP), which  
91 is responsible for the transfer of cholesterol ester and triglycerides between lipoproteins <sup>(25)</sup>  
92 and may be involved in carotenoid metabolism as well <sup>(26)</sup>.

93

## 93 Methods

94

### 95 *Subjects*

96 Results generated in this observational study were obtained from baseline values of  
97 French Caucasian subjects enrolled in the Medi-RIVAGE study <sup>(27,28)</sup>. Subjects (18 to 70  
98 years old) were recruited at the Center for Detection and Prevention of Arteriosclerosis at La  
99 Timone University Hospital (Marseille, France). The Medi-RIVAGE protocol was in  
100 accordance with the ethical standards and was approved by the regional ethics committee on  
101 human subjects in Marseille. Characteristics and nutrient intakes of the subjects are given in a  
102 recently published paper <sup>(18)</sup>.

103

### 104 *Choice of candidate SNPs*

105 Candidate SNPs were selected through analysis of previous studies describing  
106 associations between genetic polymorphisms and lipid digestion, transport or metabolism.  
107 Genotyping of apo C-III <sup>(29)</sup>, CETP <sup>(30,31)</sup>, I-FABP <sup>(32)</sup>, MTP <sup>(33)</sup>, and HL <sup>(34)</sup> was performed  
108 by using PCR amplification followed by enzymatic digestion (restriction isotyping). Note  
109 that, in the case of MTP, a mismatched primer was used to create a polymorphic site relative  
110 to the polymorphism (C in capital letter in the reverse primer in Table 1) <sup>(33)</sup>. Details on  
111 SNPs, primers and restriction enzymes are given in **Table 1**.

112

### 113 *Vitamin and carotenoid extraction and HPLC analysis*

114 Vitamin E ( $\alpha$  and  $\gamma$ -tocopherol) and carotenoids ( $\alpha$  and  $\beta$ -carotene, lutein, lycopene,  
115  $\beta$ -cryptoxanthin and zeaxanthin) were extracted from fasting plasma samples as follow:  
116 plasma were deproteinized by adding one volume of ethanol containing the internal standard  
117 (tocol for vitamin E and echinenone for carotenoids). Micronutrients were extracted twice by  
118 the addition of two volumes of hexane. All extractions were performed at room temperature  
119 under yellow light to minimize light-induced damage.  $\alpha$ -tocopherol,  $\gamma$ -tocopherol and tocol  
120 were separated using a 250  $\times$  4.6 nm reverse-phase C<sub>18</sub>, 5  $\mu$ m Zorbax column (Interchim,  
121 Montluçon, France) and a guard column. The mobile phase was 100% methanol. Carotenoids  
122 were separated using a 150 X 4.6 mm, RP C<sub>18</sub>, 3- $\mu$ m Nucleosil column (Interchim,  
123 Montluçon, France) coupled with a 250 X 4.6 mm C<sub>18</sub>, 5- $\mu$ m Hypersil guard column. The  
124 mobile phase consisted in acetonitrile/methanol containing 50 mmol/L ammonium  
125 acetate/water/dichloromethane (70/15/5/10; V/V/V/V). Tocopherols were detected at 325 nm

126 after light excitation at 292 nm, and were identified by retention time compared with pure (>  
127 97%) standards purchased from Fluka (Vaulx-en-Velin, France). Carotenoids were detected  
128 at 450 nm and identified by retention time compared with pure (> 95%) standards, which  
129 were generously donated by DSM Ltd (Basel, Switzerland). For more details see Borel et al.  
130 (<sup>18</sup>).

131

## 132 *Plasma lipids and apolipoproteins*

133 Triglycerides and total cholesterol concentrations in fasting plasma were determined  
134 by enzymatic procedures with commercial kits (Boehringer Mannheim, Meylan, France).  
135 High-density lipoprotein (HDL) cholesterol was measured after sodium phosphotungstate–  
136 magnesium chloride precipitation. Low-density lipoprotein (LDL) cholesterol was estimated  
137 indirectly by use of the Friedewald formula. Serum apolipoproteins (apo) A-I, B and E were  
138 assayed by immunonephelometry using commercial kits (Behring Werke AG, Marburg,  
139 Germany) on a BN100 nephelometer.

140

## 141 *Statistics*

142 The values cited in the text are means  $\pm$  SD. All statistical tests were performed using  
143 the SAS/STAT software package (version 9.1.3, SAS Institute, Raleigh, USA). The Gaussian  
144 distribution of dependent variables was tested using the Kolmogorov-Smirnov test. The  
145 variable was converted into logarithm 10 when the null hypothesis of test was rejected. When  
146 the distribution was too far from the normal distribution, the variable was converted into  
147 logarithm 10. Before testing the effect of genotypes on the dependent variables, interfering  
148 co-variables (adjustment factors) were identified by two approaches. In the first approach,  
149 each dependent variable was tested in univariate general linear models with the following  
150 independent qualitative variables: physical activity (3 ranges), anti-hypertensive treatment,  
151 tobacco (three levels: never a smoker, currently a smoker, a former smoker) and menopausal  
152 status. Under the second approach, linear Pearson's correlations were run between the  
153 dependent variables and the quantitative co-variables, BMI and alcohol intake, and any  
154 correlations significant at a *P* value of 0.05 were retained. Co-variables identified by either  
155 one of the two methods were included as adjustment factors for testing genotype effect. Age  
156 was always included in the adjustment.

157 The effects of the genotypes on the dependent variables (i.e. plasma levels of vitamin  
158 E and carotenoids) were tested systematically for the whole subject population and for men  
159 and women separately, using univariate general linear models. Results include adjusted *P*



160 values, non-adjusted means and SD. Interactions of genotype by gender were tested. When  
161 the effects of the genotypes differed according to gender, results are given separately for men  
162 and women. In some cases the number of subjects bearing a particular genotype was too  
163 small to find a significant association. In that case associations with pooled genotypes  
164 (subjects carrier of at least one allele versus subjects not carrier of that allele) were tested.  
165 Concerning differences in plasma concentrations of micronutrients, statistical significances  
166 were accepted when P was lower than 0.05.

167



## 167 Results

168

### 169 *Subject characteristics and nutrient intakes*

170 One hundred twenty eight subjects were enrolled in the study. Their physical  
171 characteristics, fasting plasma vitamin E and carotenoid concentrations, as well as their  
172 nutrient intakes are detailed in previous papers (<sup>18,27</sup>). The most important subject  
173 characteristics are as follow (mean  $\pm$  SD): age ( $51.5 \pm 9.9$  y), BMI ( $28.7 \pm 5.0$  kg/m<sup>2</sup>), total  
174 fasting plasma cholesterol ( $6.47 \pm 0.89$  nmol/L), fasting plasma triglycerides ( $1.55 \pm 0.95$   
175 nmol/L), fasting plasma  $\alpha$ -tocopherol ( $26.4 \pm 6.6$   $\mu$ mol/L), fasting plasma  $\gamma$ -tocopherol ( $1.5 \pm$   
176  $0.6$   $\mu$ mol/L), fasting plasma  $\alpha$ -carotene ( $0.16 \pm 0.13$   $\mu$ mol/L), fasting plasma  $\beta$ -carotene ( $0.51$   
177  $\pm 0.47$   $\mu$ mol/L), fasting plasma lycopene ( $0.38 \pm 0.22$   $\mu$ mol/L), fasting plasma lutein ( $0.42 \pm$   
178  $0.27$   $\mu$ mol/L), fasting plasma  $\beta$ -cryptoxanthin ( $0.26 \pm 0.23$   $\mu$ mol/L), fasting plasma  
179 zeaxanthin ( $0.10 \pm 0.05$   $\mu$ mol/L), total daily energy intake ( $8446 \pm 2438$  KJ), daily vitamin E  
180 intake ( $10.7 \pm 5.2$  mg), daily  $\alpha$ -carotene plus  $\beta$ -carotene intake ( $4.1 \pm 3.1$  mg).

181 The frequency distribution of the genotypes in the studied population is shown in  
182 **Table 2.**

183

### 184 *SNP related to plasma levels of vitamin E and carotenoids*

185 **Table 3** is a synthetic table showing all the relationships between the studied SNPs  
186 and the plasma concentrations of the micronutrients. The main observation from this table is  
187 that three micronutrients were related to the SNP in HL:  $\gamma$ -tocopherol,  $\alpha$ -carotene and  $\beta$ -  
188 carotene. Secondly,  $\alpha$ -tocopherol concentrations were related to two SNPs, one in apo C-III  
189 and one in CETP. Thirdly, only plasma lycopene was related to the SNP in I-FABP. Finally,  
190 the plasma xanthophylls (lutein, zeaxanthin and  $\beta$ -cryptoxanthin) were not related to any of  
191 the studied SNPs (not shown in the table).

192

### 193 *Effect of the apo C-III, CETP, and HL SNPs on plasma vitamin E concentrations*

194 Women homozygous for the G allele in the *apo C-III* gene SNP had higher ( $P < 0.05$ )  
195 plasma concentrations of  $\alpha$ -tocopherol than women who carried at least one copy of the G  
196 allele (**Figure 1**). Conversely, there was no effect of this SNP on plasma  $\alpha$ -tocopherol  
197 concentrations in males. Males homozygous for the B1 allele in the CETP SNP had lower ( $P$   
198  $< 0.05$ )  $\alpha$ -tocopherol concentrations than men who carried a B2 allele (**Figure 1**). This  
199 association was not found in females. Finally, females homozygous for the T allele of the HL

200 SNP had higher ( $P < 0.05$ )  $\gamma$ -tocopherol concentrations than individuals who carried at least  
201 one copy of the C allele at this locus (**Figure 2**).

202

203 *Effect of HL SNP on plasma levels of  $\beta$ -carotene*

204 Women homozygous for the T allele in the HL SNP had higher ( $P < 0.05$ )  $\beta$ -carotene  
205 concentrations than women who carried a C allele at this locus (**Figure 2**). Conversely, men  
206 homozygous for the T allele had lower  $\beta$ -carotene concentrations than men carrying a C allele  
207 at this locus, however this association was not significant.

208

209 *Effect of HL SNP on plasma levels of  $\alpha$ -carotene*

210 Men homozygous for the T allele at the HL SNP had 70% lower ( $P < 0.05$ ) plasma  $\alpha$ -  
211 carotene concentrations than men carrying a C allele at this locus (**Figure 2**). The same effect  
212 was observed in women (-22% in those homozygous for the T allele), but this association was  
213 not statistically significant in women.

214

215 *Effect of I-FABP SNP on plasma levels of lycopene*

216 The SNP in I-FABP was only related to plasma lycopene (**Figure 3**). Females  
217 homozygous for the G allele had lower (-23%,  $P < 0.05$ ) plasma lycopene concentrations than  
218 females carrying a A allele at this locus. Conversely, there was no significant difference in  
219 plasma lycopene concentrations between males with different genotypes.

220

221 *Relationships between SNPs and plasma micronutrient levels after adjustment for cholesterol  
222 and triglycerides*

223 The fact that positive bivariate correlations were found between plasma total  
224 cholesterol and both  $\alpha$ -tocopherol ( $r = 0.484$ ,  $P < 0.001$ ) and  $\gamma$ -tocopherol ( $r = 0.186$ ,  
225  $P=0.038$ ) prompted us to test the relationships between these two forms of vitamin E and the  
226 studied polymorphisms with and without adjustment for plasma cholesterol level.  
227 Furthermore, because a positive bivariate correlation was also found between plasma  
228 triglycerides and  $\gamma$ -tocopherol ( $r = 0.197$ ,  $P=0.030$ ),  $\gamma$ -tocopherol was adjusted for both  
229 cholesterol and triglyceride levels.

230 After adjustment, the effect of the apo C-III polymorphism on  $\alpha$ -tocopherol concentrations  
231 remained significant in women ( $P=0.038$ ) and became borderline significant for the whole  
232 population ( $P=0.057$ ). The effect of the CETP polymorphism on  $\alpha$ -tocopherol in men became

233 a tendency rather than a significant association ( $P=0.085$ ). The adjustments did not notably  
234 modify the data of the associations between HL polymorphisms and plasma  $\gamma$ -tocopherol  
235 concentrations in females, which remained significant ( $P=0.048$ ).

236 There were negative bivariate correlations between plasma triglycerides and  $\alpha$ -carotene ( $r = -$   
237  $0.249$ ,  $P=0.005$ ) and  $\beta$ -carotene ( $r = -0.226$   $P=0.011$ ). The relationships between these  
238 carotenoids and the HL polymorphism were then tested with and without adjustment for  
239 triglycerides. The adjustments did not noticeably modify the data of the associations  
240 observed.

241 Therefore, on the whole, these adjustments did not markedly modify the pre-adjustment  
242 associations observed.

243

## 243 Discussion

244

245 An *in silico* search for SNPs associated with genes involved in vitamin E homeostasis  
246 has suggested that proteins involved in lipid metabolism which indirectly influence vitamin E  
247 status are highly polymorphic and so are good candidates for interindividual variability (<sup>35</sup>).  
248 In agreement with this hypothesis we have recently found that SNPs in four genes involved in  
249 lipid metabolism (i.e. *SCARB1*, *apo A-IV*, *apo B* and *apo E*) were associated with the fasting  
250 plasma concentrations of vitamin E and carotenoids (<sup>18</sup>). To extend these findings further, our  
251 aim was to study other candidate genes that may be involved in determining the plasma  
252 concentrations of vitamin E and carotenoids. The selection of the candidate genes was based  
253 on their well-known roles in the intracellular transport of lipids (MTP and I-FABP) and  
254 lipoprotein metabolism (*apo C-III*, CETP and HL). The choice of candidate SNPs was based  
255 on studies showing that these SNPs have phenotypic effects on lipid metabolism (<sup>34,36-41</sup>).

256 The main observation of this study was the association between the SNP in HL and  
257 the fasting plasma concentration of the micronutrients. This SNP was associated with the  
258 levels of three micronutrients ( $\gamma$ -tocopherol,  $\alpha$ -carotene and  $\beta$ -carotene), while the other SNPs  
259 were only associated with one micronutrient. Since the -480C $\rightarrow$ T substitution in the  
260 promoter region of HL is functional and leads to a lower HL activity (<sup>42</sup>), we suggest that this  
261 enzyme is involved in a change in triglyceride metabolism that alters the carrying capacity of  
262 the lipoproteins for the micronutrients. It is noteworthy that no association was found  
263 between a SNP in lipoprotein lipase (-93G/Asn9), the other key intravascular enzyme  
264 involved in the hydrolysis of lipoprotein triglycerides, and these micronutrients in the same  
265 cohort of subjects (<sup>18</sup>). Nevertheless, an association between another SNP in LPL (S447X)  
266 and plasma carotenoids was observed in another french cohort (<sup>43</sup>). This remind that the  
267 results of this kind of studies can be affected by the choice of the SNP and the studied  
268 population. Since HL is assumed to hydrolyze triglycerides in chylomicron remnants, IDL  
269 and HDL (<sup>22</sup>), while lipoprotein lipase is assumed to hydrolyze these lipids in chylomicrons  
270 and VLDL (<sup>22</sup>), we suggest that the associations observed were due to the fraction of  
271 micronutrients transported in IDL and/or HDL. Further experiments are required to test this  
272 hypothesis.

273 Since  $\alpha$ -tocopherol is carried exclusively by plasma lipoproteins (<sup>44</sup>), the relationship  
274 between the SNP in *apo C-III* and the plasma levels of  $\alpha$ -tocopherol was expected. In fact,  
275 fasting plasma  $\alpha$ -tocopherol concentrations have been associated with other apolipoprotein  
276 genes, including *apo A-IV* (<sup>18</sup>) and *apo E* (<sup>18,19</sup>). Apo C-III is assumed to inhibit triglyceride

277 removal from triglyceride-rich lipoproteins <sup>(21)</sup>. The S2 allele of apo C-III is related to  
278 increased mRNA expression in vivo <sup>(45)</sup>. It is therefore possible that variation in the  
279 expression of apo C-III can affect the transfer of  $\alpha$ -tocopherol, which is probably concomitant  
280 to that of triglycerides.

281 The association between the TaqIB variant in CETP and plasma  $\alpha$ -tocopherol suggests  
282 that this enzyme, which is known to transfer cholesterol esters between HDL and apo B-100  
283 lipoproteins <sup>(46)</sup>, may be involved in the transfer of this vitamin between lipoparticles. Indeed,  
284 this variant is associated with plasma CETP levels, partly because it is in linkage  
285 disequilibrium with other functional CETP promoter polymorphisms <sup>(40)</sup> which affect CETP  
286 mass concentration. Furthermore, because it has previously been shown that  $\alpha$ -tocopherol  
287 transfer between lipoproteins is mainly due to PLTP <sup>(47)</sup>, we suggest that this association is  
288 due to the transfer of  $\alpha$ -tocopherol during lipid transfer by CETP.

289 Finally, a noteworthy association between an I-FABP variant (IFABP-Thr) and  
290 plasma lycopene level was observed. This variant is associated with a modulation in fatty acid  
291 binding <sup>(32)</sup>, and it has been suggested that the threonine-encoding allele may increase  
292 absorption and/or processing of dietary fatty acids by the intestine <sup>(32)</sup>. Since this I-FABP  
293 polymorphism has been associated with plasma triglyceride-rich lipoprotein levels <sup>(48)</sup>, and  
294 because lycopene is mainly transported by these lipoproteins <sup>(49)</sup>, the most likely hypothesis  
295 may be that variation in I-FABP activity in intestinal cells induces variations in the levels of  
296 these lipoparticles, which may affect the amount of lycopene carried in the circulation.  
297 However, the fact that no relationship was found between this SNP and plasma  $\alpha$  and  $\beta$ -  
298 carotene, which are also preferentially carried by these lipoproteins <sup>(11,12)</sup>, does not support  
299 this hypothesis. Another possibility could be that I-FABP binds and carries newly absorbed  
300 lycopene in enterocytes, although there have been no studies relating to this topic, and there is  
301 no evidence that the flux of fatty acids carried by I-FABP can indirectly affect the  
302 intracellular transport of lycopene. Therefore, these hypotheses require further experiments in  
303 order to determine the correct model for lycopene transport.

304 The lack of relationship between the MTP SNP and the plasma status of the studied  
305 micronutrients was rather unexpected. This suggests that this protein, which is involved in  
306 triglyceride packaging into chylomicrons and  $\alpha$ -tocopherol secretion into chylomicrons <sup>(10)</sup>,  
307 has no major effect on the fasting plasma concentrations of these micronutrients. However,  
308 since the MTP SNP studied (-493) is located in the promoter region of the gene and can  
309 therefore modify the expression levels of the protein <sup>(33)</sup>, it is possible that the influence of  
310 this SNP can only be observed during the postprandial period when MTP controls the flux of

311 chylomicron secretion. Unfortunately, this hypothesis could not be tested using the  
312 experimental design in this study.

313 The lack of association between the fasting plasma concentrations of lutein,  
314 zeaxanthin and  $\beta$ -cryptoxanthin and all of the studied SNPs is intriguing. This result may be  
315 explained by the fact that these carotenoids, which belong to the xanthophyll subfamily, are  
316 less hydrophobic than the carotenes (lycopene,  $\beta$ -carotene and  $\alpha$ -carotene), and are probably  
317 involved in different metabolic pathways from the carotenes. Consistent with this idea,  
318 xanthophylls readily exchange between lipoproteins, while carotenes hardly exchange  
319 between lipoproteins (<sup>26</sup>). Furthermore, lutein and zeaxanthin are present at very high  
320 concentrations in the macula lutea, while carotenes can hardly be detected in this tissue (<sup>50</sup>).  
321 Only one SNP, located in ABCG5, a membrane transporter implicated in the efflux of  
322 phytosterols out of enterocytes (<sup>51</sup>), has potentially been ( $P = 0.08$ ) associated with plasma  
323 lutein level (<sup>52</sup>), but this gene was not considered in our study.

324 The results show that most of the associations observed were gender-dependent. This  
325 phenomenon has previously been observed in several association studies (<sup>18,48</sup>), and may be  
326 explained by the well-known effects of estrogens on lipoprotein metabolism (<sup>53</sup>). Further  
327 experiments are required to confirm, and provide explanations for these intriguing  
328 observations.

329 In order to verify that the differences in plasma concentrations were not due to  
330 differences in dietary intake of micronutrients rather than the studied SNP, plasma  
331 concentrations of  $\alpha$ -tocopherol and  $\beta$ -carotene were adjusted for their dietary intake (as  
332 estimated by 3-day food records and the GENI software (Micro6, Nancy, France) based on  
333 the French REGAL food database). However, the coefficient of variations (CV) of the  
334 adjusted values were about two times higher than those of the non adjusted values.  
335 Consequently, although one difference remained significant ( $\alpha$ -tocopherol and CETP), two  
336 other became non significant after this adjustment ( $\beta$ -carotene and HL and  $\alpha$ -tocopherol and  
337 apo C-III). Nevertheless the fact that this adjustment accentuated, from 36% to 143%, the  
338 differences observed between the genotype groups (data not shown), strongly support the  
339 effects of the genetic polymorphisms.

340 Note that the associations between the studied SNP and plasma concentrations of  
341 micronutrients became nonsignificant after Bonferroni correction. The Bonferroni correction  
342 is a multiple comparison correction to avoid a lot of spurious positives. However, this  
343 correction is conservative and has a risk of discarding interesting results as nonsignificant.  
344 However, the studied SNPs had published phenotypic effect on lipid parameters and are good



345 candidate SNP for affecting plasma fat soluble micronutrient concentration. Nevertheless, we  
346 acknowledge that the associations observed should be confirmed in other studies.

347 In conclusion, while considering the limits of these types of studies, our study has  
348 identified four novel genes that are potentially involved in the plasma status of vitamin E and  
349 carotenoids. An apolipoprotein (apo C-III) and two genes involved in the intravascular  
350 metabolism of lipoproteins (HL and CETP) may affect plasma tocopherol concentrations,  
351 while a protein involved in the intracellular transport of fatty acids (I-FABP) as well as HL  
352 may affect plasma carotenoid concentrations. Although the effects of the SNPs on the activity  
353 of the associated gene products are compatible with the known roles of these proteins  
354 concerned in lipoprotein metabolism and fat-soluble micronutrients, these findings should be  
355 confirmed in further studies using other populations.



Tables

Table 1: SNP data

Gene/protein	SNP	Polymorphism	Rs number	Direct primer	Reverse primer	Ref
apo C-III	Apo CIII S1/S2	C/G	*	ggtgaccgatggcttcagtt	taccagaagtgtagagagcg	(29)
CETP	CETP TaqIB	C/T	rs708272	cactagcccagaggagtgcc	ctgagcccagccgcacactaac	(31)
HL	HL C-480T	C/T	rs1800588	ggaaattctgccaaaggctgg	ggatcacctctcaatgggtc	(34)
I-FABP	IFABP-Thr	A/G	rs1799883	caggtgtaatatagtgaaaagg	ttacctgagttcagttccg	(32)
MTP	MTP-493	G/T	rs1800591	agtttcacactaaggacaatcatcta	ggatttaatttaaactgttaattcatat cCac **	(33)

\* Not referenced. \*\* Mutated primer. Apo C-III: apolipoprotein C-III; CETP: cholesterol ester transfer protein; HL: hepatic lipase; I-FABP: intestinal fatty acid binding protein; MTP: microsomal triglyceride transfer protein.

Comment citer ce document :

Borel, P., Moussa, M., Reboul, E., Lyan, B., Defoort, C., Vincent-Baudry, S., Maillot, M., Gastaldi, M., Darmon, M., Portugal, H., Lairon, D., Planells, R. (2009). Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. British Journal of

Table 2: Frequency distribution of the genotype in the sample population

SNP	Genotype	Frequency (%)
Apo CIII S1/S2	C/C	1.8
	G/C	20.1
	G/G	78.1
CETP TaqIB	B1/B1	29.0
	B1/B2	47.9
	B2/B2	23.1
HL C-480T	C/C	62.1
	C/T	32.0
	T/T	5.9
IFABP-Thr	A/A	8.9
	A/G	39.6
	G/G	51.5
MTP-493	G/G	40.8
	G/T	49.1
	T/T	10.1

Comment citer ce document :

Borel, P., Moussa, M., Reboul, E., Lyan, B., Defoort, C., Vincent-Baudry, S., Maillot, M., Gastaldi, M., Darmon, M., Portugal, H., Lairon, D., Planells, R. (2009). Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. British Journal of

Table 3: SNPs significantly related to plasma concentrations of vitamin E and carotenoids<sup>1</sup>

Gene/protein	SNP Studied	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\alpha$ -carotene	$\beta$ -carotene	lycopene
Apo C-III	S1/S2	X	-	-	-	-
CETP	TaqIB	X	-	-	-	-
HL	HL C-480T	-	X	X	X	-
I-FABP	IFABP-Thr	-	-	-	-	X
MTP	MTP-493	-	-	-	-	-

<sup>1</sup>Single nucleotide polymorphisms (SNPs) were selected based on their published phenotypic effects upon lipid metabolism. A cross indicates a significant relationship ( $P < 0.05$ ) between an SNP and the fasting plasma micronutrient concentration. Apo C-III: apolipoprotein C-III; CETP: cholesterol ester transfer protein; HL: hepatic lipase; I-FABP: intestinal fatty acid binding protein; MTP: microsomal triglyceride transfer protein.

## Figure Legends

*Figure 1:* Plasma  $\alpha$ -tocopherol concentrations for each genotype in apo C-III (panel A) and CETP (panel B). Data are means  $\pm$  SD. Note that the figure show non corrected means while statistical tests were performed on co-variable adjusted means (see statistic paragraph). Black bars: males, white bars: females. n show the number of subjects bearing the same genotype. The asterisk in panel A indicates that  $\alpha$ -tocopherol concentration was significantly higher in female carriers of the G/G genotype in apo C-III. The “+” in panel B indicates that males who carried the B2/B2 genotype in CETP had significantly higher  $\alpha$ -tocopherol concentrations than males who carried other variants. Conversely, the “-“ indicates that males with this variant had significantly lower values than males with other variants.

*Figure 2:* Plasma  $\gamma$ -tocopherol,  $\alpha$ -carotene and  $\beta$ -carotene concentrations for each genotype in hepatic lipase. Data are means  $\pm$  SD. Note that the figure show non corrected means while statistical tests were performed on co-variable adjusted means (see statistic paragraph). Black bars: males, white bars: females. n show the number of subjects bearing the same genotype. An asterisk indicates that the plasma concentration of this group of homozygous males, or females, was significantly different from the plasma concentration of the corresponding group containing carriers of the other variants.

*Figure 3:* Plasma lycopene concentrations for each I-FABP (FABP2) genotype. Data are means  $\pm$  SD. Note that the figure show non corrected means while statistical tests were performed on co-variable adjusted means (see statistic paragraph). Black bars: males, white bars: females. n show the number of subjects bearing the same genotype. An asterisk indicates that the plasma concentration of this group of homozygous females was significantly different from the plasma concentration of the females carrying other variants.

## **Conflict of interest**

Patrick Borel, Myriam Moussa, Emmanuelle Reboul, Bernard Lyan, Catherine Defoort, Stéphanie Vincent-Baudry, Matthieu Maillot, Marguerite Gastaldi, Michel Darmon, Henri Portugal, Denis Lairon and Richard Planells have no conflict of interest to declare.

## **Support**

This study was supported by the French Ministry of Research (an AQS grant plus S. Vincent-Baudry's salary), the INSERM (an IDS grant), the Provence-Alpes-Côte d'Azur Regional Council, the Bouches du Rhône General Council, CRITT-PACA, and the following companies: Rivoire & Carret Lustucru, Jean Martin, Le Cabanon, Boulangerie Coagulation Surgelés, Distplack Mariani, Minoterie Giraud.

## **Contribution of each author**

CD, MG, HP, DL and RP contributed to the design of the human study. PB, MMo, ER, MG, DL and RP choosed the candidate genes and genetic polymorphisms. SVB and HP carried out the practical aspects of the study, with help from MG. MMo and BL performed the micronutrient analysis. MMA and MD carried out the statistical analyses, and initially interpreted the data before writing the manuscript. PB wrote the first draft of the manuscript with help from MMo, ER and DL. All authors participated in the writing of the final draft of the manuscript and in the final interpretation of the data.

## References

1. Jian L, Du CJ, Lee AH, Binns CW. (2005) Do dietary lycopene and other carotenoids protect against prostate cancer? *Int J Cancer* **113**, 1010-1014.
2. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med* **113**, 71-88.
3. Johnson EJ, Chung HY, Caldarella SM, Snodderly DM. (2008) The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. *Am J Clin Nutr* **87**, 1521-1529.
4. Borel P. (2003) Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin Chem Lab Med* **41**, 979-994.
5. Reboul E, Abou L, Mikail C, Ghiringhelli O, Andre M, Gleize B, Kaloustian J, Portugal H, Amiot M, Borel P. (2003) Lutein is apparently absorbed by a carrier-mediated transport process in Caco-2 cells. *Clin Nutr* **22**, S103.
6. Reboul E, Abou L, Mikail C, Ghiringhelli O, Andre M, Portugal H, Jourdheuil-Rahmani D, Amiot MJ, Lairon D, Borel P. (2005) Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). *Biochem J* **387**, 455-461.
7. Reboul E, Klein A, Bietrix F, Gleize B, Malezet-Desmoulins C, Schneider M, Margotat A, Lagrost L, Collet X, Borel P. (2006) Scavenger receptor class B type I (SR-BI) is involved in vitamin E transport across the enterocyte. *J Biol Chem* **281**, 4739-4745.
8. van Bennekum A, Werder M, Thuahnai ST, Han CH, Duong P, Williams DL, Wettstein P, Schulthess G, Phillips MC, Hauser H. (2005) Class B Scavenger Receptor-Mediated Intestinal Absorption of Dietary beta-Carotene and Cholesterol. *Biochemistry* **44**, 4517-4525.
9. During A, Dawson HD, Harrison EH. (2005) Carotenoid Transport Is Decreased and Expression of the Lipid Transporters SR-BI, NPC1L1, and ABCA1 Is Downregulated in Caco-2 Cells Treated with Ezetimibe. *J Nutr* **135**, 2305-2312.
10. Anwar K, Iqbal J, Hussain MM. (2007) Mechanisms involved in vitamin E transport by primary enterocytes and in vivo absorption. *J Lipid Res* **48**, 2028-2038.
11. Krinsky N, Cornwell D, Oncley J. (1958) The transport of vitamin A and carotenoids in human plasma. *Arch Biochem Biophys* **73**, 233-246.
12. Romanchik JE, Morel DW, Harrison EH. (1995) Distributions of carotenoids and alpha-tocopherol among lipoproteins do not change when human plasma is incubated in vitro. *J Nutr* **125**, 2610-2617.

13. Massey JB. (1984) Kinetics of transfer of  $\alpha$ -tocopherol between model and native plasma lipoproteins. *Biochim Biophys Acta* **793**, 387-392.
14. Traber MG, Lane JC, Lagmay NR, Kayden HJ. (1992) Studies on the transfer of tocopherol between lipoproteins. *Lipids* **27**, 657-663.
15. Kostner GM, Oettl K, Jauhiainen M, Ehnholm C, Esterbauer H, Dieplinger H. (1995) Human plasma phospholipid transfer protein accelerates exchange transfer of alpha-tocopherol between lipoproteins and cells. *Biochem J* **305**, 659-667.
16. Jiang XC, Tall AR, Qin S, Lin M, Schneider M, Lalanne F, Deckert V, Desrumaux C, Athias A, et al. (2002) Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. *J Biol Chem* **277**, 31850-31856.
17. Goti D, Reicher H, Malle E, Kostner GM, Panzenboeck U, Sattler W. (1998) High-density lipoprotein (HDL3)-associated alpha-tocopherol is taken up by HepG2 cells via the selective uptake pathway and resecreted with endogenously synthesized apo-lipoprotein B-rich lipoprotein particles. *Biochem J* **332**, 57-65.
18. Borel P, Moussa M, Reboul E, Lyan B, Defoort C, Vincent-Baudry S, Maillot M, Gastaldi M, Darmon M, et al. (2007) Human plasma levels of vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid metabolism. *J Nutr* **137**, 2653-2659.
19. Gomez-Coronado D, Entrala A, Alvarez JJ, Ortega H, Olmos JM, Castro M, Sastre A, Herrera E, Lasuncion MA. (2002) Influence of apolipoprotein E polymorphism on plasma vitamin A and vitamin E levels. *Eur J Clin Invest* **32**, 251-258.
20. Ortega H, Castilla P, Gomez-Coronado D, Garces C, Benavente M, Rodriguez-Artalejo F, de Oya M, Lasuncion MA. (2005) Influence of apolipoprotein E genotype on fat-soluble plasma antioxidants in Spanish children. *Am J Clin Nutr* **81**, 624-632.
21. Fruchart JC, Duriez P. (1995) [The important role of apolipoprotein C-III in lipoprotein metabolism]. *C R Seances Soc Biol Fil* **189**, 889-897.
22. Santamarina-Fojo S, Haudenschild C. (2000) Role of hepatic and lipoprotein lipase in lipoprotein metabolism and atherosclerosis: studies in transgenic and knockout animal models and somatic gene transfer. *Int J Tissue React* **22**, 39-47.
23. Besnard P, Niot I, Poirier H, Clement L, Bernard A. (2002) New insights into the fatty acid-binding protein (FABP) family in the small intestine. *Mol Cell Biochem* **239**, 139-147.
24. Yamashita S. (2001) [Microsomal triglyceride transfer protein (MTP)]. *Nippon Rinsho* **59 Suppl 2**, 226-235.
25. Oliveira HCF, Ma LM, Milne R, Marcovina SM, Inazu A, Mabuchi H, Tall AR. (1997) Cholesteryl ester transfer protein activity enhances plasma cholesteryl ester formation - Studies in CETP transgenic mice and human genetic CETP deficiency. *Arterioscler Thromb Vasc Biol* **17**, 1045-1052.



26. Tyssandier V, Choubert G, Grolier P, Borel P. (2002) Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins. *Int J Vitam Nutr Res* **72**, 300-308.
27. Vincent S, Gerber M, Bernard MC, Defoort C, Loundou A, Portugal H, Planells R, Juhan-Vague I, Charpiot P, et al. (2004) The Medi-RIVAGE study (Mediterranean Diet, Cardiovascular Risks and Gene Polymorphisms): rationale, recruitment, design, dietary intervention and baseline characteristics of participants. *Public Health Nutr* **7**, 531-542.
28. Vincent-Baudry S, Defoort C, Gerber M, Bernard MC, Verger P, Helal O, Portugal H, Planells R, Grolier P, et al. (2005) The Medi-RIVAGE study: reduction of cardiovascular disease risk factors after a 3-mo intervention with a Mediterranean-type diet or a low-fat diet. *Am J Clin Nutr* **82**, 964-971.
29. Salas J, Jansen S, Lopez-Miranda J, Ordovas JM, Castro P, Marin C, Ostos MA, Bravo MD, Jimenez-Perez J, et al. (1998) The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an oral-glucose-tolerance test after consumption of a diet rich in saturated fats. *Am J Clin Nutr* **68**, 396-401.
30. Drayna D, Lawn R. (1987) Multiple RFLPs at the human cholesteryl ester transfer protein (CETP) locus. *Nucleic Acids Res* **15**, 4698.
31. Fumeron F, Betoulle D, Luc G, Behague I, Ricard S, Poirier O, Jemaa R, Evans A, Arveiler D, et al. (1995) Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *J Clin Invest* **96**, 1664-1671.
32. Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, Mochizuki H, Bennett PH, Bogardus C, Prochazka M. (1995) An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest* **95**, 1281-1287.
33. Karpe F, Lundahl B, Ehrenborg E, Eriksson P, Hamsten A. (1998) A common functional polymorphism in the promoter region of the microsomal triglyceride transfer protein gene influences plasma LDL levels. *Arterioscler Thromb Vasc Biol* **18**, 756-761.
34. Jansen H, Chu G, Ehnholm C, Dallongeville J, Nicaud V, Talmud PJ. (1999) The T allele of the hepatic lipase promoter variant C-480T is associated with increased fasting lipids and HDL and increased preprandial and postprandial LpCIII:B : European Atherosclerosis Research Study (EARS) II. *Arterioscler Thromb Vasc Biol* **19**, 303-308.
35. Doring F, Rimbach G, Lodge JK. (2004) In silico search for single nucleotide polymorphisms in genes important in vitamin E homeostasis. *IUBMB Life* **56**, 615-620.
36. Zampino R, Ingrosso D, Durante-Mangoni E, Capasso R, Tripodi MF, Restivo L, Zappia V, Ruggiero G, Adinolfi LE. (2008) Microsomal triglyceride transfer protein (MTP) -493G/T gene polymorphism contributes to fat liver accumulation in HCV genotype 3 infected patients. *J Viral Hepat*, in press.

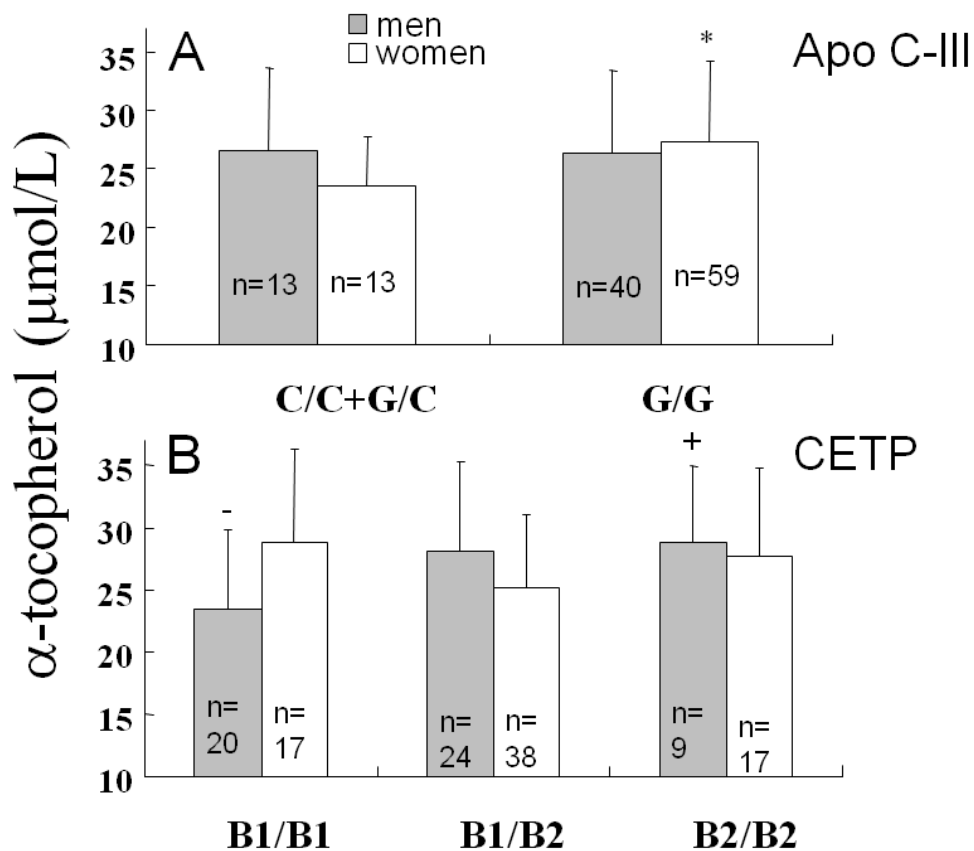
37. Bjorn L, Leren TP, Ose L, Hamsten A, Karpe F. (2000) A functional polymorphism in the promoter region of the microsomal triglyceride transfer protein (MTP -493G/T) influences lipoprotein phenotype in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* **20**, 1784-1788.
38. Georgopoulos A, Bloomfield H, Collins D, Brousseau ME, Ordovas JM, O'Connor JJ, Robins SJ, Schaefer EJ. (2007) Codon 54 polymorphism of the fatty acid binding protein (FABP) 2 gene is associated with increased cardiovascular risk in the dyslipidemic diabetic participants of the Veterans Affairs HDL intervention trial (VA-HIT). *Atherosclerosis* **194**, 169-174.
39. Espino-Montoro A, Barrios-Artillo M, Lopez-Chozas JM, Cayuela A, Stiefel P, Villar J. (2003) Influence of polymorphism (RFLP-sstI) at the apolipoprotein C-III gene locus on the lipoprotein metabolism and insulin resistance in essential hypertensive patients. Interaction between gender and genetic polymorphism. *Nutr Metab Cardiovasc Dis* **13**, 194-201.
40. Frisdal E, Klerkx AH, Le Goff W, Tanck MW, Lagarde JP, Jukema JW, Kastelein JJ, Chapman MJ, Guerin M. (2005) Functional interaction between -629C/A, -971G/A and -1337C/T polymorphisms in the CETP gene is a major determinant of promoter activity and plasma CETP concentration in the REGRESS Study. *Hum Mol Genet* **14**, 2607-2618.
41. McCaskie PA, Cadby G, Hung J, McQuillan BM, Chapman CM, Carter KW, Thompson PL, Palmer LJ, Beilby JP. (2006) The C-480T hepatic lipase polymorphism is associated with HDL-C but not with risk of coronary heart disease. *Clin Genet* **70**, 114-121.
42. Botma GJ, Verhoeven AJ, Jansen H. (2001) Hepatic lipase promoter activity is reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by Upstream Stimulatory Factor. *Atherosclerosis* **154**, 625-632.
43. Herbeth B, Gueguen S, Leroy P, Siest G, Visvikis-Siest S. (2007) The lipoprotein lipase serine 447 stop polymorphism is associated with altered serum carotenoid concentrations in the Stanislas Family Study. *J Am Coll Nutr* **26**, 655-662.
44. Behrens WA, Madere R. (1985) Transport of  $\alpha$  and  $\gamma$ -tocopherol in human plasma lipoproteins. *Nutr Res* **5**, 167-174.
45. Esterbauer H, Hell E, Krempler F, Patsch W. (1999) Allele-specific differences in apolipoprotein C-III mRNA expression in human liver. *Clin Chem* **45**, 331-339.
46. Barter P. (2000) CETP and atherosclerosis. *Arterioscler Thromb Vas Biol* **20**, 2029-2031.
47. Klein A, Deckert V, Schneider M, Dutrillaux F, Hammann A, Athias A, Le Guern N, Pais de Barros JP, Desrumaux C, et al. (2006) Alpha-tocopherol modulates phosphatidylserine externalization in erythrocytes: relevance in phospholipid transfer protein-deficient mice. *Arterioscler Thromb Vasc Biol* **26**, 2160-2167.
48. Gastaldi M, Dizière S, Defoort C, Portugal H, Lairon D, Darmon M, Planells R. (2007) Sex-specific association of fatty acid binding protein 2 and microsomal triacylglycerol transfer protein variants with response to dietary lipid changes in the 3-mo Medi-RIVAGE primary intervention study. *Am J Clin Nutr* **86**, 1633-1641.

49. Clevidence BA, Bieri JG. (1993) Association of carotenoids with human plasma lipoproteins. *Methods Enzymol* **214**, 33-46.
50. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J. (1993) Stereochemistry of the human macular carotenoids. *Invest Ophthalmol Vis Sci* **34**, 2033-2040.
51. Duan LP, Wang HH, Ohashi A, Wang DQ. (2006) Role of intestinal sterol transporters Abcg5, Abcg8, and Npc111 in cholesterol absorption in mice: gender and age effects. *Am J Physiol Gastrointest Liver Physiol* **290**, G269-276.
52. Herron KL, McGrane MM, Waters D, Lofgren IE, Clark RM, Ordovas JM, Fernandez ML. (2006) The ABCG5 polymorphism contributes to individual responses to dietary cholesterol and carotenoids in eggs. *J Nutr* **136**, 1161-1165.
53. Henriksson P, Stamberger M, Eriksson M, Rudling M, Diczfalusy U, Berglund L, Angelin B. (1989) Oestrogen-induced changes in lipoprotein metabolism: role in prevention of atherosclerosis in the cholesterol-fed rabbit. *Eur J Clin Invest* **19**, 395-403.

# Postprint

Version définitive du manuscrit publié dans / Final version of the manuscript published in : British Journal of Nutrition, 2008, Ahead of print, DOI:

Figure 1



Comment citer ce document :

Borel, P., Moussa, M., Reboul, E., Lyan, B., Defoort, C., Vincent-Baudry, S., Maillot, M., Gastaldi, M., Darmon, M., Portugal, H., Lairon, D., Planells, R. (2009). Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. British Journal of

Figure 2

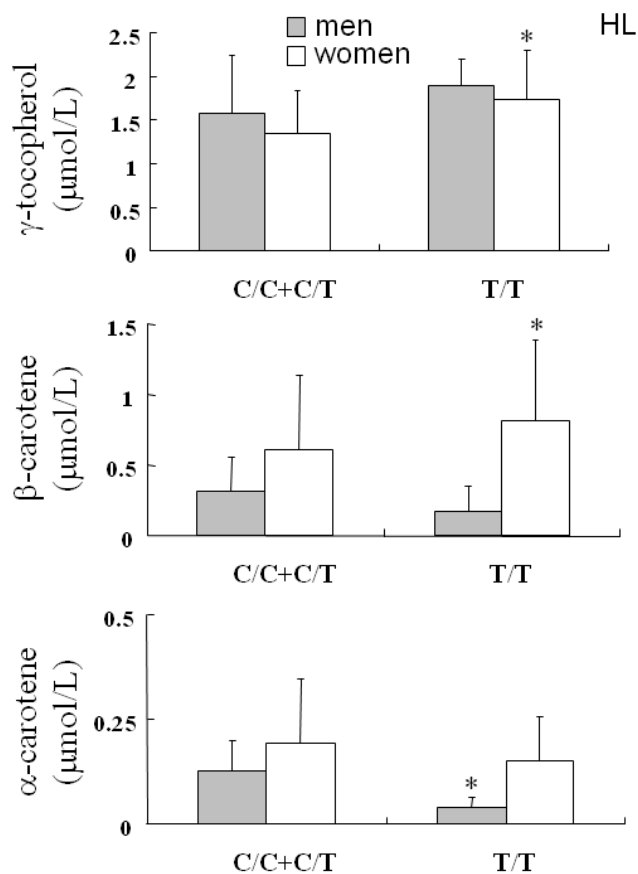
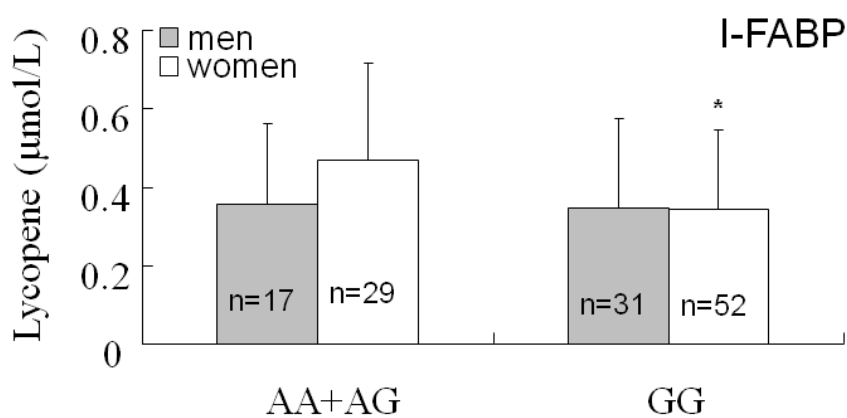


Figure 3



Comment citer ce document :

Borel, P., Moussa, M., Reboul, E., Lyan, B., Defoort, C., Vincent-Baudry, S., Maillot, M., Gastaldi, M., Darmon, M., Portugal, H., Lairon, D., Planells, R. (2009). Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. British Journal of