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

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

25

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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 (α- and γ-tocopherol) and six carotenoids (α and

54 β-carotene, β-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 [1], cardio-vascular disease [2] and eye disease [3]. 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 [4]. Recent

60 studies have established that the intestinal absorption of these compounds involves the class B

61 type I scavenger receptor (SR-BI) [5-9]. 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 α-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 [10]. 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 [11,12]. 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 [13,14] is mediated by

75 phospholipid transfer protein [15,16], and SR-BI is involved in the transfer of α-tocopherol

76 from HDL to tissues [17]. 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 [18-20], 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 ([21]). 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   ([22]). The third gene encodes I-FABP, which is involved in the intracellular transport of fatty

88   acids in the small intestine ([23]). The fourth gene encodes a protein in charge of the

89   incorporation of triglycerides in chylomicrons, microsomal triglyceride transfer protein

90   (MTP) ([24]). 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 ([25])

92   and may be involved in carotenoid metabolism as well ([26]).

93

Methods

Subjects

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

Choice of candidate SNPs

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

Vitamin and carotenoid extraction and HPLC analysis

Vitamin E (α and γ-tocopherol) and carotenoids (α and β-carotene, lutein, lycopene, β-cryptoxanthin and zeaxanthin) were extracted from fasting plasma samples as follow: plasma were deproteinized by adding one volume of ethanol containing the internal standard (tocol for vitamin E and echinenone for carotenoids). Micronutrients were extracted twice by the addition of two volumes of hexane. All extractions were performed at room temperature under yellow light to minimize light-induced damage. α-tocopherol, γ-tocopherol and tocol were separated using a 250 $\times$ 4.6 nm reverse-phase C_{18}, 5 μm Zorbax column (Interchim, Montluçon, France) and a guard column. The mobile phase was 100% methanol. Carotenoids were separated using a 150 X 4.6 mm, RP C_{18}, 3-μm Nucleosil column (Interchim, Montluçon, France) coupled with a 250 X 4.6 mm C_{18}, 5-μm Hypersil guard column. The mobile phase consisted in acetonitrile/methanol containing 50 mmol/L ammonium 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 ([18]).

131

132 *Plasma lipids and apoliporoteins*

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 ± 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.

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C-III, CETP, hepatic lipase, intestinal fatty acid binding protein and MTP. British Journal of

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 ([18,27]). The most important subject
173 characteristics are as follow (mean ± SD): age (51.5 ± 9.9 y), BMI (28.7 ± 5.0 kg/m^2), total
174 fasting plasma cholesterol (6.47 ± 0.89 nmol/L), fasting plasma triglycerides (1.55 ± 0.95
175 nmol/L), fasting plasma α-tocopherol (26.4 ± 6.6 μmol/L), fasting plasma γ-tocopherol (1.5 ±
176 0.6 μmol/L), fasting plasma α-carotene (0.16 ± 0.13 μmol/L), fasting plasma β-carotene (0.51
177 ± 0.47 μmol/L), fasting plasma lycopene (0.38 ± 0.22 μmol/L), fasting plasma lutein (0.42 ±
178 0.27 μmol/L), fasting plasma β-cryptoxanthin (0.26 ± 0.23 μmol/L), fasting plasma
179 zeaxanthin (0.10 ± 0.05 μmol/L), total daily energy intake (8446 ± 2438 KJ), daily vitamin E
180 intake (10.7 ± 5.2 mg), daily α-carotene plus β-carotene intake (4.1 ± 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: γ-tocopherol, α-carotene and β-
188 carotene. Secondly, α-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 β-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 α-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 α-tocopherol
197 concentrations in males. Males homozygous for the B1 allele in the CETP SNP had lower (P
198 < 0.05) α-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 ($P < 0.05$) γ-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 β-carotene*

204 Women homozygous for the T allele in the HL SNP had higher ($P < 0.05$) β-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 β-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 α-carotene*

210 Men homozygous for the T allele at the HL SNP had 70% lower ($P < 0.05$) plasma α-

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 α-tocopherol (r = 0.484, $P < 0.001$) and γ-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 γ-tocopherol (r = 0.197, $P=0.030$), γ-tocopherol was adjusted for both

229 cholesterol and triglyceride levels.

230 After adjustment, the effect of the apo C-III polymorphism on α-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 α-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 γ-tocopherol

235 concentrations in females, which remained significant (P=0.048).

236 There were negative bivariate correlations between plasma triglycerides and α-carotene (r = -

237 0.249, P=0.005) and β-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 [35].

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 [18]. 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 [34,36-41].

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 (γ-tocopherol, α-carotene and β-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 [42], 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 [18]. Nevertheless, an association between another SNP in LPL (S447X)

266 and plasma carotenoids was observed in another french cohort [43]. 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 [22], while lipoprotein lipase is assumed to hydrolyze these lipids in chylomicrons

270 and VLDL [22], 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 α-tocopherol is carried exclusively by plasma lipoproteins [44], the relationship

274 between the SNP in apo C-III and the plasma levels of α-tocopherol was expected. In fact,

275 fasting plasma α-tocopherol concentrations have been associated with other apoliprotein

276 genes, including *apo A-IV* [18] and *apo E* [18,19]. Apo C-III is assumed to inhibit triglyceride

277 removal from triglyceride-rich lipoproteins ([21]). The S2 allele of apo C-III is related to

278 increased mRNA expression in vivo ([45]). It is therefore possible that variation in the

279 expression of apo C-III can affect the transfer of α-tocopherol, which is probably concomitant

280 to that of triglycerides.

281 The association between the TaqIB variant in CETP and plasma α-tocopherol suggests

282 that this enzyme, which is known to transfer cholesterol esters between HDL and apo B-100

283 lipoproteins ([46]), 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 disequelibrium with other functional CETP promoter polymorphisms ([40]) which affect CETP

286 mass concentration. Furthermore, because it has previously been shown that α-tocopherol

287 transfer between lipoproteins is mainly due to PLTP ([47]), we suggest that this association is

288 due to the transfer of α-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 ([32]), and it has been suggested that the threonine-encoding allele may increase

292 absorption and/or processing of dietary fatty acids by the intestine ([32]). Since this I-FABP

293 polymorphism has been associated with plasma triglyceride-rich lipoprotein levels ([48]), and

294 because lycopene is mainly transported by these lipoproteins ([49]), 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 α and β-

298 carotene, which are also preferentially carried by these lipoproteins ([11,12]), 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 α-tocopherol secretion into chylomicrons ([10]),

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 ([33]), 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 β-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, β-carotene and α-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 ([26]). Furthermore, lutein and zeaxanthin are present at very high

320 concentrations in the macula lutea, while carotenes can hardly be detected in this tissue ([50]).

321 Only one SNP, located in ABCG5, a membrane transporter implicated in the efflux of

322 phytosterols out of enterocytes ([51]), has potentially been ($P = 0.08$) associated with plasma

323 lutein level ([52]), 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 ([18,48]), and may be

326 explained by the well-known effects of estrogens on lipoprotein metabolism ([53]). 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 α-tocopherol and β-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 (α-tocopherol and CETP), two

336 other became non significant after this adjustement (β-carotene and HL and α-tocopherol and

337 apo C-III). Nevertheless the fact that this adjustement 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	taccagaaggtggatagagcg	[29]
CETP	CETP TaqIB	C/T	rs708272	cactagcccagaggagtgcc	ctgagcccagccgcacactaac	[31]
HL	HL C-480T	C/T	rs1800588	ggaaattctgccaaaggtcgg	ggatcacctctcaatgggtc	[34]
I-FABP	IFABP-Thr	A/G	rs1799883	caggtgttaatatagtgaaaagg	ttaccctgagttcagttccg	[32]
MTP	MTP-493	G/T	rs1800591	agtttcacactaaggacaatcatcta	ggatttaaatttaaactgttaattcatat 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.

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

Table 3: SNPs significantly related to plasma concentrations of vitamin E and carotenoids[1]

Gene/protein	SNP Studied	α-tocopherol	γ-tocopherol	α-carotene	β-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	-	-	-	-	-

[1]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 α-tocopherol concentrations for each genotype in apo C-III (panel A) and CETP (panel B). Data are means ± 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 α-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 α-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 γ-tocopherol, α-carotene and β-carotene concentrations for each genotype in hepatic lipase. Data are means ± 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 ± 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.

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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 chose 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.

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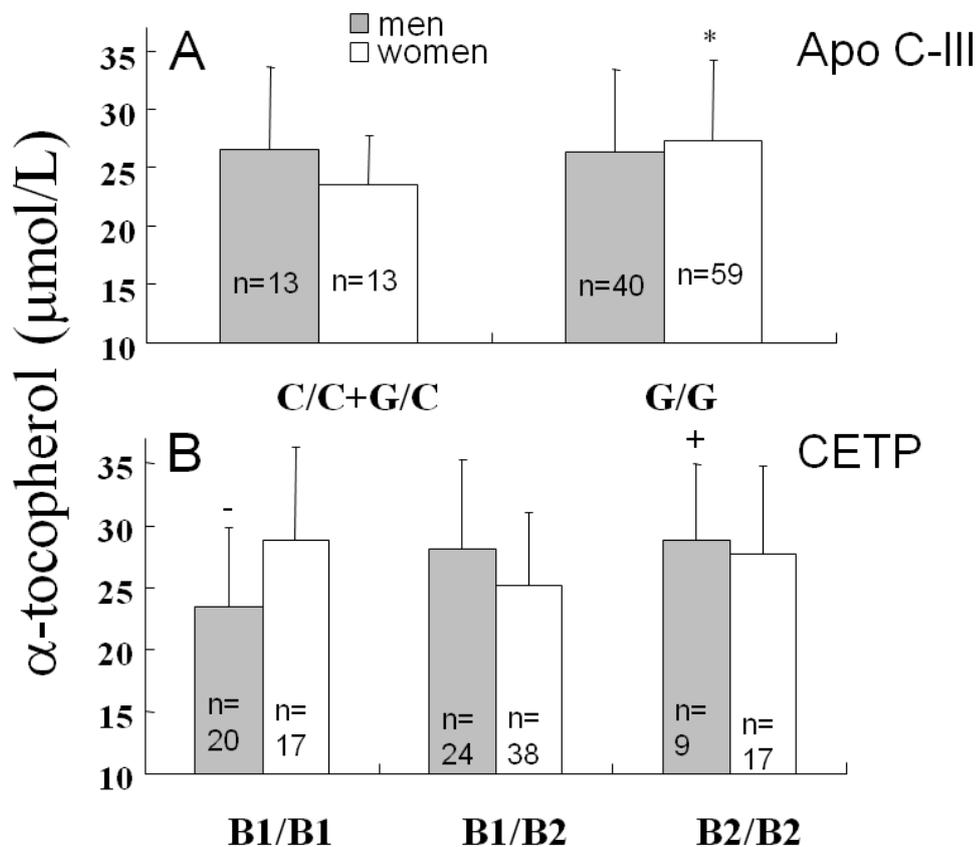
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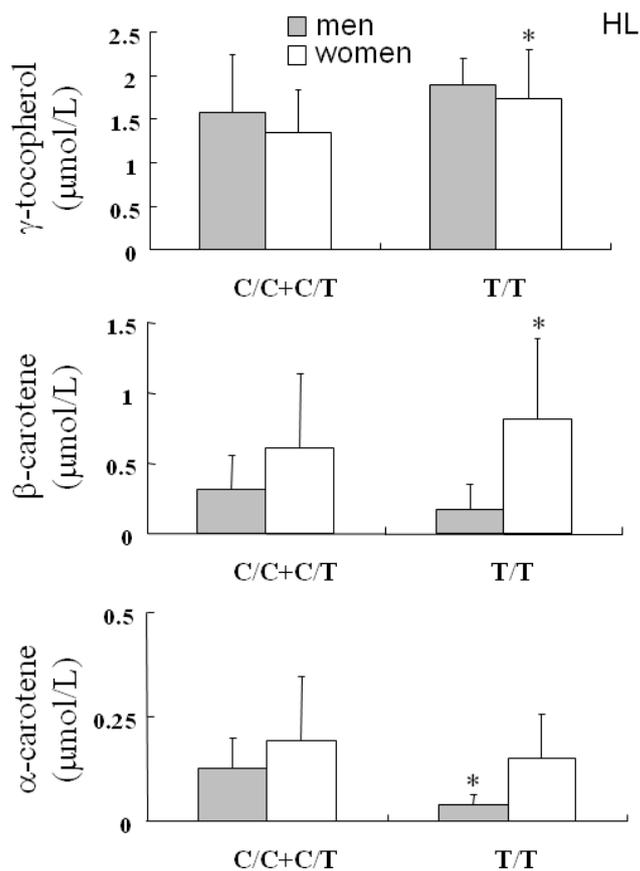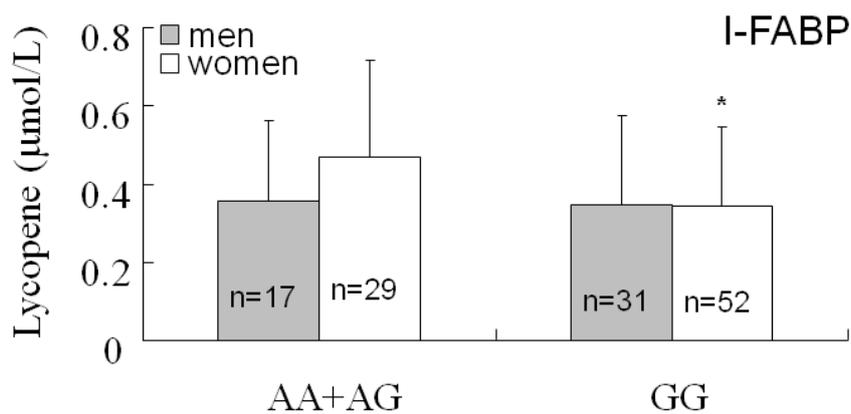
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