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

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

Abstract

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

Introduction

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

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

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 × 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

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

Plasma lipids and apoliporoteins

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

Statistics

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

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

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

Results

Subject characteristics and nutrient intakes

One hundred twenty eight subjects were enrolled in the study. Their physical characteristics, fasting plasma vitamin E and carotenoid concentrations, as well as their nutrient intakes are detailed in previous papers ([18,27]). The most important subject characteristics are as follow (mean ± SD): age (51.5 ± 9.9 y), BMI (28.7 ± 5.0 kg/m^2), total fasting plasma cholesterol (6.47 ± 0.89 nmol/L), fasting plasma triglycerides (1.55 ± 0.95 nmol/L), fasting plasma α-tocopherol (26.4 ± 6.6 µmol/L), fasting plasma γ-tocopherol (1.5 ± 0.6 µmol/L), fasting plasma α-carotene (0.16 ± 0.13 µmol/L), fasting plasma β-carotene (0.51 ± 0.47 µmol/L), fasting plasma lycopene (0.38 ± 0.22 µmol/L), fasting plasma lutein (0.42 ± 0.27 µmol/L), fasting plasma β-cryptoxanthin (0.26 ± 0.23 µmol/L), fasting plasma zeaxanthin (0.10 ± 0.05 µmol/L), total daily energy intake (8446 ± 2438 KJ), daily vitamin E intake (10.7 ± 5.2 mg), daily α-carotene plus β-carotene intake (4.1 ± 3.1 mg).

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

SNP related to plasma levels of vitamin E and carotenoids

Table 3 is a synthetic table showing all the relationships between the studied SNPs and the plasma concentrations of the micronutrients. The main observation from this table is that three micronutrients were related to the SNP in HL: γ-tocopherol, α-carotene and β-carotene. Secondly, α-tocopherol concentrations were related to two SNPs, one in apo C-III and one in CETP. Thirdly, only plasma lycopene was related to the SNP in I-FABP. Finally, the plasma xanthophylls (lutein, zeaxanthin and β-cryptoxanthin) were not related to any of the studied SNPs (not shown in the table).

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

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

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

Effect of HL SNP on plasma levels of β-carotene

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

Effect of HL SNP on plasma levels of α-carotene

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

Effect of I-FABP SNP on plasma levels of lycopene

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

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

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

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

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

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

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

Discussion

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

The main observation of this study was the association between the SNP in HL and the fasting plasma concentration of the micronutrients. This SNP was associated with the levels of three micronutrients (γ-tocopherol, α-carotene and β-carotene), while the other SNPs were only associated with one micronutrient. Since the -480C→T substitution in the promoter region of HL is functional and leads to a lower HL activity ([42]), we suggest that this enzyme is involved in a change in triglyceride metabolism that alters the carrying capacity of the lipoproteins for the micronutrients. It is noteworthy that no association was found between a SNP in lipoprotein lipase (-93G/Asn9), the other key intravascular enzyme involved in the hydrolysis of lipoprotein triglycerides, and these micronutrients in the same cohort of subjects ([18]). Nevertheless, an association between another SNP in LPL (S447X) and plasma carotenoids was observed in another french cohort ([43]). This remind that the results of this kind of studies can be affected by the choice of the SNP and the studied population. Since HL is assumed to hydrolyze triglycerides in chylomicron remnants, IDL and HDL ([22]), while lipoprotein lipase is assumed to hydrolyze these lipids in chylomicrons and VLDL ([22]), we suggest that the associations observed were due to the fraction of micronutrients transported in IDL and/or HDL. Further experiments are required to test this hypothesis.

Since α-tocopherol is carried exclusively by plasma lipoproteins ([44]), the relationship between the SNP in apo C-III and the plasma levels of α-tocopherol was expected. In fact, fasting plasma α-tocopherol concentrations have been associated with other apoliprotein genes, including *apo A-IV* ([18]) and *apo E* ([18,19]). Apo C-III is assumed to inhibit triglyceride

removal from triglyceride-rich lipoproteins ([21]). The S2 allele of apo C-III is related to increased mRNA expression in vivo ([45]). It is therefore possible that variation in the expression of apo C-III can affect the transfer of α-tocopherol, which is probably concomitant to that of triglycerides.

The association between the TaqIB variant in CETP and plasma α-tocopherol suggests that this enzyme, which is known to transfer cholesterol esters between HDL and apo B-100 lipoproteins ([46]), may be involved in the transfer of this vitamin between lipoparticles. Indeed, this variant is associated with plasma CETP levels, partly because it is in linkage disequelibrium with other functional CETP promoter polymorphisms ([40]) which affect CETP mass concentration. Furthermore, because it has previously been shown that α-tocopherol transfer between lipoproteins is mainly due to PLTP ([47]), we suggest that this association is due to the transfer of α-tocopherol during lipid transfer by CETP.

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

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

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

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

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

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

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

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

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

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Figure 1

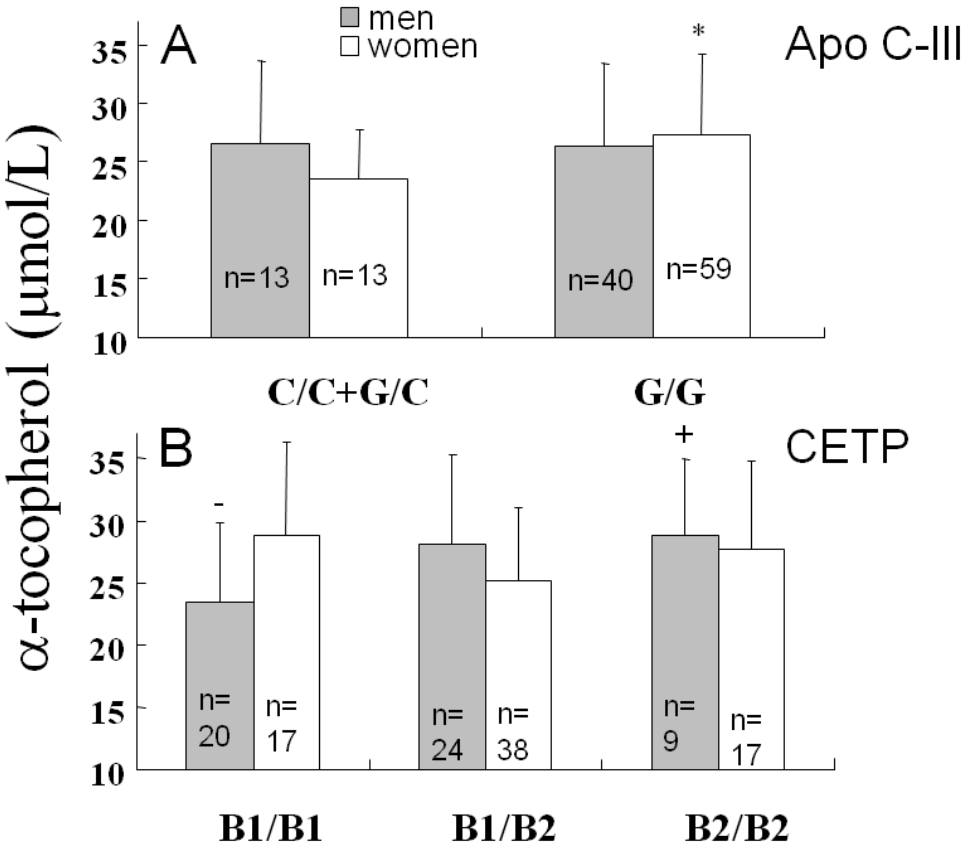

Figure 2

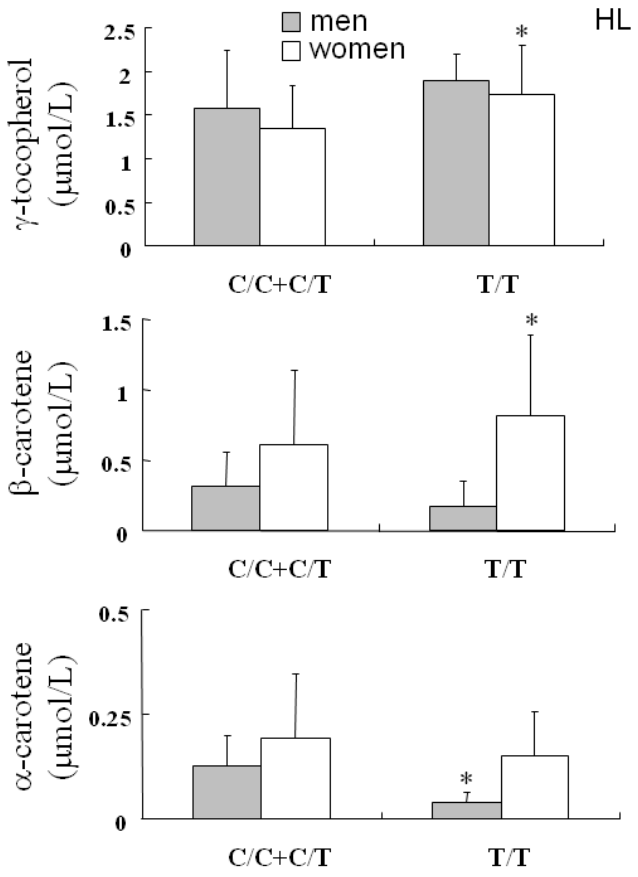

Figure 3