Human fasting plasma concentrations of vitamin E and carotenoids, and their association with genetic variants in apo C-III, cholesteryl ester transfer protein, hepatic lipase, intestinal fatty acid binding protein and microsomal triacylglycerol transfer protein

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Plasma concentrations of vitamin E and carotenoids are governed by several factors, including genetic factors. Single nucleotide polymorphisms (SNP) 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 SNP 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 129 French Caucasian subjects (forty-eight males and eighty-one females). Candidate SNP were genotyped by PCR amplification followed by restriction fragment length polymorphisms. Plasma γ-tocopherol, α-carotene and β-carotene concentrations were significantly different ($P < 0.05$) in subjects who carried different SNP variants in hepatic lipase. Plasma α-tocopherol concentrations were significantly different in subjects who had different SNP variants in apo C-III and cholesteryl ester transfer protein (CETP). Plasma lycopene concentrations were significantly different ($P < 0.05$) in women who had different SNP variants in intestinal fatty acid binding protein (I-FABP). Finally, there was no effect of SNP variants in microsomal TAG transfer protein upon the plasma concentrations of these micronutrients. Most of the observed differences remained significant after the plasma micronutrients were adjusted for plasma TAG 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.

Carotene: Intestinal fatty acid binding protein: Lycopene: Single nucleotide polymorphisms: Tocopherols

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], CVD[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 (intestinal fatty acid binding protein (I-FABP) and liver FABP) are likely candidates. Part of the mechanism involved in α-tocopherol incorporation in chylomicrons has been recently elucidated. The microsomal TAG transfer protein (MTP) results in a significant decrease in the secretion of vitamin E with TAG-rich lipoproteins and underscores the importance of the chylomicron pathway in net

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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 (SNP) in some genes encoding these proteins have been found to be related to the fasting plasma concentrations of these micronutrients[18–20], we designed the present study to assess whether SNP 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 apo C-III, which inhibits TAG removal from the plasma[21]. The second gene encodes hepatic lipase (HL), which along with lipoprotein lipase, is responsible for the lipolysis of lipoprotein TAG 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 TAG in chylomicrons, MTP[24]. The fifth gene encodes a protein, cholesteryl ester transfer protein (CETP), which is responsible for the transfer of cholesteryl ester and TAG 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 (aged 18–70 years) were recruited at the Centre 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 single nucleotide polymorphisms

Candidate SNP 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 enzymic digestion (restriction isotyping). Note that, in the case of MTP, a mismatched primer was used to create a polymorphic site relative to the polymorphism (capital letter C in the reverse primer in Table 1)[33]. Details on SNP, 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 follows: plasma was deproteinised 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 minimise light-induced damage. α-Tocopherol, γ-tocopherol and tocol were separated using a 250×4.6 nm reverse-phase C_{18}, $5 \mu m$ Zorbax column (Interchim, Montluçon, France) and a guard column. The mobile phase was 100 % methanol. Carotenoids were separated using a 150×4.6 mm, RP C_{18}, $3 \mu m$ Nucleosil column (Interchim, Montluçon, France) coupled with a 250×4.6 mm C_{18}, $5 \mu m$ Hypersil guard column. The mobile phase consisted of acetonitrile–methanol containing 50 mM-ammonium acetate–water–dichloromethane (70/15/5/10, by vol.). 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 apolipoproteins

TAG and total cholesterol concentrations in fasting plasma were determined by enzymic procedures with commercial kits (Boehringer Mannheim, Meylan, France). HDL-cholesterol was measured after sodium phosphotungstate–magnesium chloride precipitation. LDL-cholesterol was estimated indirectly by use of the Friedewald formula. Serum apo A-I, B and E were assayed

Table 1. Single nucleotide polymorphism (SNP) data

Gene/protein	SNP	Polymorphism	Rs number	Direct primer	Reverse primer	Reference
Apo C-III	Apo CIII S1/S2	C/G	*	ggtgaccgatggcttcagtt	taccagaaggtggatagagcg	Salas et al.[29]
CETP	CETP TaqIB	C/T	rs708272	cactagcccagaggagtgcc	ctgagcccagccgcacactaac	Fumeron et al.[31]
HL	HL C-480T	C/T	rs1800588	ggaaattctgccaaaggtcgg	ggatcacctctcaatgggtc	Jansen et al.[34]
I-FABP	IFABP-Thr	A/G	rs1799883	caggtgttaatatagtgaaaagg	ttaccctgagttcagttccg	Baier et al.[32]
MTP	MTP-493	G/T	rs1800591	agtttcacactaaggacaatcatcta	ggatttaaatttaaactgttaattcatatcCac†	Karpe et al.[33]

CETP, cholesteryl ester transfer protein; HL, hepatic lipase; I-FABP, intestinal fatty acid binding protein; MTP, microsomal TAG transfer protein.
* Not referenced.
† Mutated primer.

by immunonephelometry using commercial kits (Behring Werke AG, Marburg, Germany) on a BN100 nephelometer.

Statistics

The values cited in the text are mean values and standard deviations. All statistical tests were performed using the SAS/ STAT software package (version 9.1.3; SAS Institute, Cary, NC, USA). The Gaussian distribution of dependent variables was tested using the Kolmogorov–Smirnov test. The variable was logarithm 10-converted when the null hypothesis of the test was rejected. Before testing the effect of genotypes on the dependent variables, interfering covariables (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 (three 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 covariables, BMI and alcohol intake, and any correlations significant at a P value of 0·05 were retained. Covariables identified by either one of the two methods were included as adjustment factors for testing the effect of genotype. 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 mean values and standard deviations. Interactions of genotype by sex were tested. When the effects of the genotypes differed according to sex, 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 *v.* subjects not carrier of that allele) were tested. Concerning differences in plasma concentrations of micronutrients, statistical significance was accepted when P was less than 0·05.

Results

Subject characteristics and nutrient intakes

One hundred and twenty-nine 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 were as follows: age (51·5 (SD 9·9) years), BMI (28·7 (SD 5·0) kg/m^2), total fasting plasma cholesterol (6·47 (SD 0·89) nmol/l), fasting plasma TAG (1·55 (SD 0·95) nmol/l), fasting plasma α-tocopherol (26·4 (SD 6·6) μmol/l), fasting plasma γ-tocopherol (1·5 (SD 0·6) μmol/l), fasting plasma α-carotene (0·16 (SD 0·13) μmol/l), fasting plasma β-carotene (0·51 (SD 0·47) μmol/l), fasting plasma lycopene (0·38 (SD 0·22) μmol/l), fasting plasma lutein (0·42 (SD 0·27) μmol/l), fasting plasma β-cryptoxanthin (0·26 (SD 0·23) μmol/l), fasting plasma zeaxanthin (0·10 (SD 0·05) μmol/l), total daily energy intake (8446 (SD 2438) kJ), daily vitamin E intake (10·7 (SD 5·2) mg) and daily α-carotene plus β-carotene intake (4·1 (SD 3·1) mg).

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

Single nucleotide polymorphisms related to plasma levels of vitamin E and carotenoids

Table 3 is a synthetic table showing all the relationships between the studied SNP 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. Second, α-tocopherol concentrations were related to two SNP, one in apo C-III and one in CETP. Third, 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 SNP (not shown in the Table).

Effect of the apo C-III, cholesteryl ester transfer protein and hepatic lipase single nucleotide polymorphisms 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 (Fig. 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 (Fig. 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 (Fig. 2).

Effect of hepatic lipase single nucleotide polymorphisms 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

Table 2. Frequency distribution of 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
I-FABP-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

SNP, single nucleotide polymorphism; CETP, cholesteryl ester transfer protein; HL, hepatic lipase; I-FABP, intestinal fatty acid binding protein; MTP, microsomal TAG transfer protein.

Table 3. Single nucleotide polymorphisms (SNP) significantly related to plasma concentrations of vitamin E and carotenoids†

Gene/protein	SNP studied	α-Tocopherol	γ-Tocopherol	α-Carotene	β-Carotene	Lycopene
Apo C-III	S1/S2	*	–	–	–	–
CETP	TaqIB	*	–	–	–	–
HL	HL C-480T	–	*	*	*	–
I-FABP	IFABP-Thr	–	–	–	–	*
MTP	MTP-493	–	–	–	–	–

CETP, cholesteryl ester transfer protein; HL, hepatic lipase; I-FABP, intestinal fatty acid binding protein; MTP, microsomal TAG transfer protein.
* Significant relationship ($P < 0.05$) between an SNP and the fasting plasma micronutrient concentration.
† SNP were selected based on their published phenotypic effects upon lipid metabolism.

carried a C allele at this locus (Fig. 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 hepatic lipase single nucleotide polymorphisms 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 (Fig. 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.
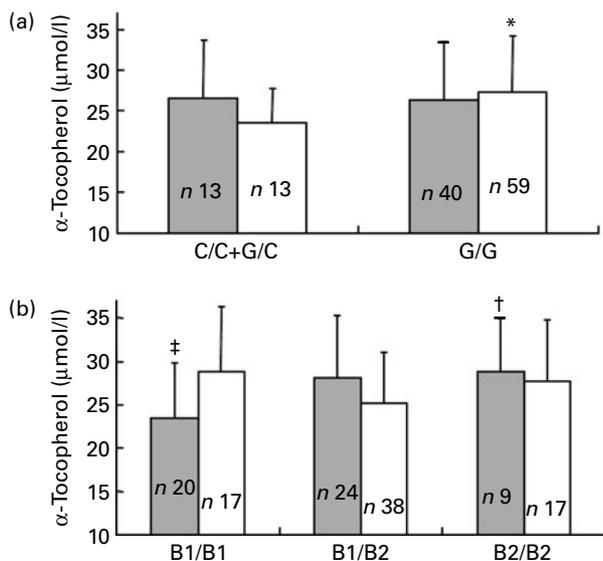

Fig. 1. Plasma α-tocopherol concentrations for each genotype in apo C-III (a) and cholesteryl ester transfer protein (CETP) (b). Data are means, with standard deviations represented by vertical bars. Note that the charts show non-corrected means, while statistical tests were performed on covariable-adjusted means (see the Statistics section). (■), Males; (□), females. The numbers of subjects bearing the same genotype are shown. * Mean value was significantly higher in female carriers of the G/G genotype than female carriers of the C/C + G/C genotypes. † Mean value was significantly higher in male carriers of the B2/B2 genotype than males who carried other variants. ‡ Mean value was significantly lower in male carriers of the B1/B1 genotype than males with other variants.

Effect of intestinal fatty acid binding protein single nucleotide polymorphisms on plasma levels of lycopene

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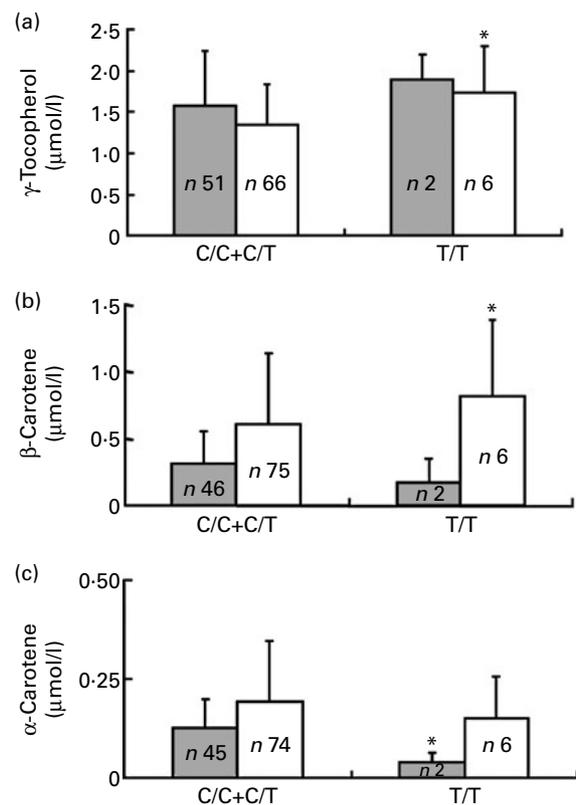

Fig. 2. Plasma γ-tocopherol (a), β-carotene (b) and α-carotene (c) concentrations for each genotype in hepatic lipase. Data are means, with standard deviations represented by vertical bars. Note that the charts show non-corrected means, while statistical tests were performed on covariable-adjusted means (see the Statistics section). (■), Males; (□), females. The numbers of subjects bearing the same genotype are shown. * Mean value of this group of homozygous males, or females, was significantly different from that of the corresponding group containing carriers of the other variants.
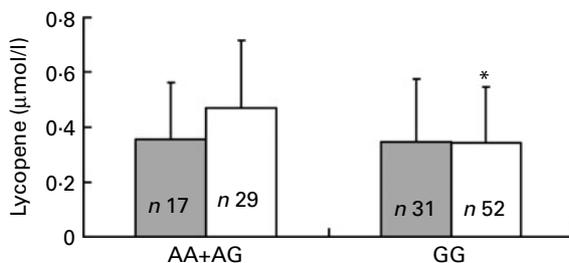
Fig. 3. Plasma lycopene concentrations for each intestinal fatty acid binding protein (FABP2) genotype. Data are means, with standard deviations represented by vertical bars. Note that the charts show non-corrected means, while statistical tests were performed on covariable-adjusted means (see the Statistics section). (▨), Males; (▢), females. The numbers of subjects bearing the same genotype are shown. * Mean value was significantly different from that of the females carrying the other variants.

Relationships between single nucleotide polymorphisms and plasma micronutrient levels after adjustment for cholesterol and triacylglycerols

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 TAG and γ-tocopherol (r 0·197; $P = 0·030$), γ-tocopherol was adjusted for both cholesterol and TAG 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 TAG 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 TAG. 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 SNP 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 SNP 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 SNP was based on studies showing that these SNP have phenotypic effects on lipid metabolism[34,36–41].

The main observation of the present 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 SNP were only associated with one micronutrient. Since the $-480\text{C} \rightarrow \text{T}$ 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 TAG 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 ($-93\text{G}/\text{Asn9}$), the other key intravascular enzyme involved in the hydrolysis of lipoprotein TAG, 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 is a reminder that the results of this kind of study can be affected by the choice of the SNP and the studied population. Since HL is assumed to hydrolyse TAG in chylomicron remnants, intermediate-density lipoprotein (IDL) and HDL[22], while lipoprotein lipase is assumed to hydrolyse 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 apo genes, including *apo A-IV*[18] and *apo E*[18,19]. Apo C-III is assumed to inhibit TAG removal from TAG-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 TAG.

The association between the TaqIB variant in CETP and plasma α-tocopherol suggests that this enzyme, which is known to transfer cholesteryl 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 (I-FABP-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 TAG-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 TAG 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 the present study.

The lack of association between the fasting plasma concentrations of lutein, zeaxanthin and β-cryptoxanthin and all of the studied SNP 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 the present study.

The results show that most of the associations observed were sex-dependent. This phenomenon has previously been observed in several association studies[18,48], and may be explained by the well-known effects of oestrogens 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 d food records and GENI software (Micro6, Nancy, France) based on the French REGAL food database). However, the 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 others became non-significant after this adjustment (β-carotene and HL and α-tocopherol and apo C-III). Nevertheless the fact that this adjustment accentuated, from 36 % to 143 %, the differences observed between the genotype groups (data not shown) strongly support the effects of the genetic polymorphisms.

It is noteworthy that the associations between the studied SNP and plasma concentrations of micronutrients became non-significant after Bonferroni correction. The Bonferroni correction is a multiple comparison correction to avoid spurious positives. However, this correction is conservative and has a risk of discarding interesting results as non-significant. However, the studied SNP had published phenotypic effects 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, the present 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 SNP 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.

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