Polymorphisms in the *CD36/FAT* gene are associated with plasma vitamin E concentrations in humans[1–3]

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ABSTRACT

Background: Blood vitamin E concentrations are modulated by dietary, metabolic, and genetic factors. CD36 (cluster of differentiation 36), a class B scavenger receptor, might be involved in tissue vitamin E uptake and thus would influence blood vitamin E concentrations.

Objective: The goal of the study was to assess the association between *CD36* single nucleotide polymorphisms (SNPs) and plasma α-tocopherol concentrations in humans.

Design: A subsample from the adult SU.VI.MAX (SUpplementation en VItamines et Minéraux AntioXydants) cohort ($n = 621$) and the adolescent cross-sectional HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study ($n = 993$) were genotyped for *CD36* SNPs (4 and 10 SNPs, respectively). Fasting plasma α-tocopherol concentrations were assayed by using HPLC. Associations were determined by haplotype analyses and by general linear regression models.

Results: In the SU.VI.MAX subsample, haplotype analyses showed that some haplotypes of SNPs rs1984112, rs1527479, rs7755, and rs1527483 tended to be associated with plasma α-tocopherol concentrations ($P = 0.08$ and $P = 0.09$ for haplotypes 1222 and 1122, respectively). We then investigated the whole known common genetic variability (10 SNPs) of *CD36* in the HELENA Study. Three SNPs were associated with lower plasma α-tocopherol concentrations (rs1984112: -3.2%, $P = 0.053$; rs1761667: -2.9%, $P = 0.046$; rs1527479: -3.7%, $P = 0.0061$). After correction for multiple testing, the association between rs1527479 and α-tocopherol concentrations remained significant. This association was modulated by concentrations of fasting serum triglycerides (P for interaction $= 0.006$) and long-chain polyunsaturated fatty acids (P for interaction $= 0.005$).

Conclusion: Our results suggest that CD36 can modulate blood α-tocopherol concentrations and may therefore be involved in the intestinal absorption or tissue uptake of vitamin E. *Am J Clin Nutr* 2011;93:644–51.

INTRODUCTION

Vitamin E is the main fat-soluble antioxidant in the human diet. Dietary vitamin E comprises 4 *RRR*-tocopherols (α, β, γ, and δ) and 4 *RRR*-tocotrienols (α, β, γ, and δ). Alpha- and γ-tocopherols are the main forms consumed in Western countries. α-Tocopherol has the highest biological activity of all vitamin E forms because, after hepatic uptake, the α-tocopherol transfer protein (α-TTP) mediates the selective resecretion of α-tocopherol from the liver into plasma (1). Recent studies have shown that, in addition to the well-established antioxidant properties of vitamin E, this group of compounds can also modulate gene expression (2–7), inflammation, and cell signaling. Together with the long-standing debate on the preventive role of vitamin E in cardiovascular disease (8–11), these new biological properties have emphasized the need to gain a better understanding of the factors affecting this vitamin's absorption and tissue distribution.

Over the past few decades, the intestinal absorption of dietary vitamin E has been considered to be a passive process. The tissue distribution of vitamin E was assumed to be driven by lipoproteins: LDL was providing tissues with vitamin E, whereas HDL was participating in the reverse transport of vitamin E from the tissues to the liver (12). However, recent studies have broken

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this paradigm by showing that the scavenger receptor class B type I (SR-BI, previously known to mediate the transfer of cholesterol from HDL into cells) is involved in both α-tocopherol uptake by human intestinal cells (13) and its distribution to several specific tissues (including brain, ovary, lung, and testis) in mice (14).

SR-BI is not the only class B scavenger receptor present in human tissues; CD36 [cluster of differentiation 36, also known as fatty acid translocase (FAT)] plays a key role in fatty acid (FA) metabolism and is expressed in intestinal and other tissues (notably adipose tissue and the spleen) (15–17). Given that SR-BI and CD36 share many structural characteristics (2 transmembrane domains and one extracellular loop), locate to the same specific membrane microdomain (the caveolae), and both have a broad substrate specificity, we hypothesized that CD36 might also be involved in vitamin E metabolism.

In such a case, blood concentrations of vitamin E would (at least in part) be under the control of CD36. Indeed, blood concentrations of vitamin E can be modulated by the dietary intake of vitamin E, by oxidative stress (that may depletes vitamin E) (18), and by polymorphisms in genes involved in the metabolism of vitamin E and lipoproteins (19–27). Several candidate gene association studies showed that polymorphisms in genes involved in lipoprotein transport, such as *APOE*, *APOA4*, *APOC3*, *hepatic lipase*, *SCARB1*, *CETP*, *TTPA*, and *SEC14L2* were associated with circulating vitamin E concentrations (19, 22–25). These results were recently strengthened by the result of a genome-wide association study (GWAS) showing an association between an *APOA5* polymorphism and circulating α-tocopherol concentrations (26).

The aim of the present study was to assess whether *CD36* polymorphisms were associated with blood concentrations of α-tocopherol in 2 independent populations: a subsample of the SU.VI.MAX (SUpplementation en VItamines et Minéraux Anti-oXydants) French adult study cohort (*n* = 621) (28) and the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Europe-wide adolescent cross-sectional study (*n* = 993) (29).

SUBJECTS AND METHODS

The SU.VI.MAX Study

Subjects

SU.VI.MAX was a randomized, double-blind, placebo-controlled, primary-prevention trial designed to test the effect of supplemented nutritional doses of antioxidants on the incidence of cancer and coronary heart diseases (28, 30). A total of 13,017 subjects (5141 men aged 45–60 y and 7876 women aged 35–60 y were included in the study between October 1994 and June 1995. The mean follow-up period lasted 7.5 y.

From this cohort, 4497 subjects were randomly selected and genotyped for several SNPs in 14 candidate genes potentially involved in the metabolism of carotenoids and vitamin E. A subsample of 621 subjects were then selected to obtain groups with contrasted haplotypes (to improve the chance to observe significant differences) in whom plasma α-tocopherol was measured (ie, the final sample studied in the present study). The characteristics of this subsample are shown in **Table 1**.

Biochemical measures

Venous blood samples were obtained after a 12-h fast, and biochemical assays of α-tocopherol and cholesterol were per-

TABLE 1
Characteristics of the study subjects[1]

	SU.VI.MAX Study (*n* = 621)	HELENA Study (*n* = 993)
Sex (*n*)		
Male	281	477
Female	340	516
Age (y)	61.6 ± 6.3[2]	14.8 ± 1.4
BMI (kg/m^2)	25.8 ± 4.1	21.2 ± 3.6
α-Tocopherol (μmol/L)	28.96 ± 6.96	22.98 ± 4.90
Total cholesterol (mmol/L)	5.75 ± 0.95	4.17 ± 0.70
α-Tocopherol:cholesterol (μmol/mmol)	5.08 ± 1.03	5.54 ± 0.91

[1] SU.VI.MAX, SUpplementation en VItamines et Minéraux AntioXydants; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence.
[2] Mean ± SD (all such values).

formed in the INRA UMR1260 laboratory in Marseille. Plasma concentrations of tocopherol were measured by using HPLC as follows. Tocopherol and tocol were separated with a 250 × 4.6 mm reversed-phase Zorbax C_{18} 5 μm column (Interchim, Montluçon, France) and a guard column. The mobile phase was 100% methanol, the flow rate was 1.5 mL/min, and the column was kept at a constant temperature (30°C). The HPLC system consisted of a Dionex separation module (with a P680 HPLC pump and an ASI-100 automated sample injector; Dionex, Aix-en-Provence, France) and a fluorimetric detector (Jasco, Nantes, France). The compounds were detected by emission at 325 nm (after excitation at 292 nm) and identified by their retention time relative to pure (>95%) standards. Quantification was performed with Chromeleon software (version 6.50 SP4 Build 1000; Dionex) by comparing peak areas with standard reference curves. All solvents used were HPLC grade (SDS, Peypin, France). Plasma tocopherol concentrations are in accordance with data reported in the literature and within the range of normality, which indicated that the storage conditions (−80°C) were adequate. The total cholesterol concentration was measured in an enzymatic assay (Advia 1650; Bayer Diagnostics, New York, NY).

Genomic DNA was prepared from 2 mL whole blood and purified with the NucleoSpin Blood kit (Macherey Nagel, Hoerdt, France). An average of 15 μg DNA was isolated from each blood sample. The purity and quantity of DNA was checked by spectrophotometry at 260 and 280 nm. A total of 100 μL DNA at a concentration of 10 ng/μL was added to a genotyping plate (Dutscher, Marseille, France).

SNP selection and genotyping

In the SU.VI.MAX cohort, 4 candidate SNPs in *CD36* (rs1984112, rs1527479, rs7755, and rs1527483) were selected through a review of the literature on associations with phenotypes related to lipid metabolism (31, 32). The SNP panel was further validated for the oligoligation assay (SNPlex, *see* below) by applying the following criteria: *1*) genome screening, in which the SNPs may be located within a genome region that is homologous with at least one other genome region, leading to a lack of assay specificity and the potential for spurious ligation templates; *2*) assay rules, in which a valid individual SNP assay cannot be designed because of detrimental sequence contexts or

nonoptimal interactions between the assay components (certain characteristics of the SNP sequence or assay components may create secondary structures and reduce assay performance, eg, a series of contiguous *G* alleles or a series of 16 contiguous weak *A* or *T* bases within 25 bases of the SNP); and *3*) pooling rules, in which there may be detrimental interactions between specific SNP assays in the assay pools and thus the generation of false signals due to interaction with genomic DNA. SNPs were genotyped in an oligoligation assay (SNPlex; Applied Biosystems, Foster City, CA) (33, 34) according to the manufacturers' instructions.

The HELENA Study

Subjects

The recruitment and phenotyping of the adolescents in the HELENA cross-sectional study (www.helenastudy.com) were described in detail elsewhere (29) (*see* Supplementary Text 1 under "Supplemental data" in the online issue). Briefly, a total of 3865 adolescents were recruited between 2006 and 2007. Data were collected at a total of 10 centers in 9 European countries. Subjects were randomly selected from schools by using a proportional cluster sampling method, and age was taken into account. One-third of the classes were randomly selected for blood collection ($n = 1155$). The body mass index (BMI) and a blood α-tocopherol measurement were available for 993 adolescents (ie, the final sample in the present study).

The data were recorded on a detailed case report form in accordance with standardized procedures. At each center, trained researchers carried out comprehensive physical examinations, including weight, height, and blood pressure measurements. The protocol was approved by the appropriate investigational review board for each investigating center. Written informed consent was obtained from each adolescent and both of his or her parents or legal representatives. Participation in the study was voluntary. The subjects' clinical characteristics are presented in Table 1.

Biochemical measures

Venous blood samples were drawn after a 10-h overnight fast. Blood samples were sent to a central laboratory (the Institut für Ernährungs-und Lebensmittelwissenschaften analytic laboratory at the University of Bonn, Bonn, Germany). α-Tocopherol was analyzed by HPLC (Sykam, Gilching, Germany). Separation was carried out on a LiChrosper Si 100 RP-18 column (125 × 4 mm, 5 μm; Merck, Darmstadt, Germany) and with an isocratic mobile phase. The mobile phase consisted of hexane/isopropanol (98/2; wt/wt). The flow rate was 2 mL/min, and the ultraviolet wavelength for detection was 292 nm (ultraviolet-Vis 205; Merck). Plasma tocopherol concentrations were in accordance with data reported in the literature and within the range of normality. Serum total cholesterol was measured enzymatically with the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) by using the manufacturer's reagents and instructions. Serum triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol were enzymatically assayed with the Dimension RxL clinical chemistry system. After Folch extraction, the serum phospholipid fraction was separated by using thin-layer chromatography to measure FAs by gas chromatography as previously described (35). FAs were ex-

pressed as a percentage area by integrating the area under the peak and dividing the results by the total area for all FAs. The CVs were <4.4% for all FA analyses. The long-chain polyunsaturated FAs (LC-PUFAs) corresponded to the sum of polyunsaturated FAs (PUFAs) with ≥20 carbons and 2 double bonds (composed at 75% by n−6 arachidonic acid and n−3 docosahexaenoic acid).

Blood for DNA extraction was collected into EDTA-containing K3 tubes and stored at the Institut für Ernährungs-und Lebensmittelwissenschaften and then sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille (Lille, France). DNA was extracted from white blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) and stored at −20°C.

SNP selection and genotyping

With the criteria used in our SNP selection procedure [a minor allele frequency (MAF) > 0.05 and tagSNPs with an r^2 value > 0.8], the November 2008 release 24 of the HapMap database described 42 SNPs in the *CD36* gene locus, which can be captured by 7 tagSNPs. Indeed, 5 large SNP blocks and 2 SNPs were in low linkage disequilibrium (LD) with the others. In the present study, we selected one SNP from each block (rs3211931 from block 1, tagging 9 other SNPs; rs1527479 from block 2, tagging 9 other SNPs; rs3211816 from block 3, tagging 8 other SNPs; rs3211867 from block 4, tagging 8 other SNPs; and rs1527483 from block 5, tagging one other SNP) and the 2 independent SNPs (rs3211883 and rs3211908). To cover the whole genetic variability of *CD36*, we also included 3 tagSNPs as described by Ma et al (31) that were not present in the HapMap database (rs1984112, rs1761667, and rs1049673). The subjects' samples were genotyped on an Illumina system; VeraCode technology was used for 1 SNP (rs1761667), and GoldenGate technology was used for the 9 others (http://illumina.com). The average genotyping success rate was 99.4%.

Haplotype analysis

In the SU.VI.MAX Study, only haplotype analyses were feasible because the subjects were selected on haplotypes and were performed by using the Thesias software package (http://ecgene.net/genecanvas) (36) (adjusted for age, sex, and BMI).

In the HELENA Study, haplotype frequencies derived from all of the studied polymorphisms were first estimated independently of any phenotype. On the basis of the inferred haplotype structure and the $2^K - 1$ possible combinations of 1 to K polymorphisms, we computed a minimal set of polymorphisms (referred to as HtSNPs), which was sufficient for characterizing all haplotypes with a frequency >0.01 (as in Tregouet et al; 37). These HtSNPs were then used to test for associations between *CD36* gene haplotypes and blood α-tocopherol concentrations. To reduce the haplotype dimension and select the most informative and parsimonious haplotype configuration when predicting phenotypic variability, we applied the maximum likelihood model to all the possible 1 to k-loci combinations of polymorphisms, which could be derived from the set of k HtSNPs. For each model (including one with no polymorphisms), an Akaike information criterion (AIC) (38) was calculated. All AIC values were rescaled by subtracting the smallest AIC value obtained in the whole set of models. According to a rule derived by extensive Monte Carlo

TABLE 2
Effect of the main *CD36* haplotypes on α-tocopherol concentrations compared with that of the most frequent haplotype in the SU.VI.MAX (SUpplementation en VItamines et Minéraux AntioXydants) Study cohort[1]

Haplotype	*n*	Frequency	Haplotype additive effect (95% CI)[2]	*P*
			μmol/mmol	
1111	277	0.452	4.56 ± 1.06 (reference)	—
2221	156	0.253	0.059 (−0.084, 0.203)	0.42
1121	44	0.072	0.106 (−0.144, 0.357)	0.41
2211	43	0.069	0.056 (−0.161, 0.274)	0.61
1222	29	0.048	0.220 (−0.023, 0.463)	0.08
1221	22	0.034	0.175 (−0.132, 0.482)	0.26
1122	17	0.028	0.314 (−0.047, 0.676)	0.09
1211	14	0.022	−0.118 (−0.757, 0.521)	0.72
2222	6	0.010	−0.053 (−1.110, 1.004)	0.92

[1] Means and *P* values for the α-tocopherol:total cholesterol ratios were calculated and adjusted for age, sex, and BMI. Single nucleotide polymorphisms in the following order were used: rs1984112, rs1527479, rs7755, and rs1527483. Only haplotypes with a frequency ≥1% are indicated. 1 = major allele; 2 = minor allele.

[2] Values are the difference in means (95% CIs) compared with the reference haplotype (1111; mean ± SD) obtained with Thesias software (http://ecgene.net/genecanvas).

simulation, all models with a rescaled AIC ≤ 2 could be considered as "equivalent" to the model with the lowest minAIC. The most parsimonious of the latter models (corresponding to the minimal haplotype configuration) was selected.

Other statistical methods

The LD was assessed by using Haploview software (http://www.broadinstitute.org/haploview/haploview). Statistical analyses were performed with SAS software (SAS Institute Inc, Cary, NC). Departure from Hardy-Weinberg equilibrium within the study groups was evaluated by using a chi-square test with 1 df. Fasting serum triglyceride concentrations were skewed and were normalized by logarithmic transformation in all analyses. α-Tocopherol values were divided by total cholesterol values before the statistical analysis, to take lipid concentrations into account. For the sake of clarity, nontransformed values of α-tocopherol and triglycerides are presented.

The association between genotypes and α-tocopherol concentrations was estimated with a general linear regression model (GLM) in HELENA using dominant and recessive models (except when the SNP's minor allele frequency was too low) (SAS

TABLE 3
α-Tocopherol concentrations according to genotype distributions of *CD36* single nucleotide polymorphisms (SNPs) in the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study[1]

SNP	Genotype			*P* (dominant)[2]	*P* (recessive)[2]
	11	12	22		
rs1984112				0.32	0.053
Genotype	*AA* (383)	*AG* (456)	*GG* (154)		
α-Tocopherol:total cholesterol	5.59 ± 0.89[3]	5.55 ± 0.94	5.39 ± 0.80		
rs1761667				0.99	0.046
Genotype	*AA* (264)	*AG* (481)	*GG* (218)		
α-Tocopherol:total cholesterol	5.54 ± 0.88	5.59 ± 0.93	5.41 ± 0.85		
rs1527479				0.72	0.0061
Genotype	*GG* (278)	*GA* (493)	*AA* (222)		
α-Tocopherol:total cholesterol	5.56 ± 0.89	5.61 ± 0.94	5.38 ± 0.83		
rs3211816				0.61	0.14
Genotype	*GG* (388)	*GA* (441)	*AA* (135)		
α-Tocopherol:total cholesterol	5.57 ± 0.89	5.57 ± 0.95	5.43 ± 0.81		
rs3211867				0.66	NC
Genotype	*CC* (866)	*CA* (121)	*AA* (6)		
α-Tocopherol:total cholesterol	5.54 ± 0.90	5.57 ± 0.96	5.57 ± 0.96		
rs3211883				0.49	NC
Genotype	*TT* (818)	*TA* (166)	*AA* (9)		
α-Tocopherol:total cholesterol	5.55 ± 0.96	5.51 ± 0.92	5.51 ± 0.92		
rs3211908				0.96	NC
Genotype	*CC* (898)	*CT* (93)	*TT* (2)		
α-Tocopherol:total cholesterol	5.54 ± 0.90	5.55 ± 0.95	5.55 ± 0.95		
rs3211931				0.36	0.10
Genotype	*CC* (291)	*CT* (499)	*TT* (203)		
α-Tocopherol:total cholesterol	5.58 ± 0.88	5.56 ± 0.94	5.45 ± 0.87		
rs1527483				0.91	NC
Genotype	*CC* (865)	*CT* (124)	*TT* (4)		
α-Tocopherol:total cholesterol	5.54 ± 0.90	5.58 ± 0.95	5.58 ± 0.95		
rs1049673				0.28	0.13
Genotype	*GG* (288)	*GC* (501)	*CC* (203)		
α-Tocopherol:total cholesterol	5.59 ± 0.88	5.55 ± 0.93	5.45 ± 0.88		

[1] *n* in parentheses. α-Tocopherol was measured in μmol/L and total cholesterol in mmol/L. 1 = major allele; 2 = minor allele. NC, not calculated (the minor allele frequency was too low).

[2] *P* values were calculated for the α-tocopherol:total cholesterol ratios by using a general linear model and were adjusted for age, sex, BMI, and center.
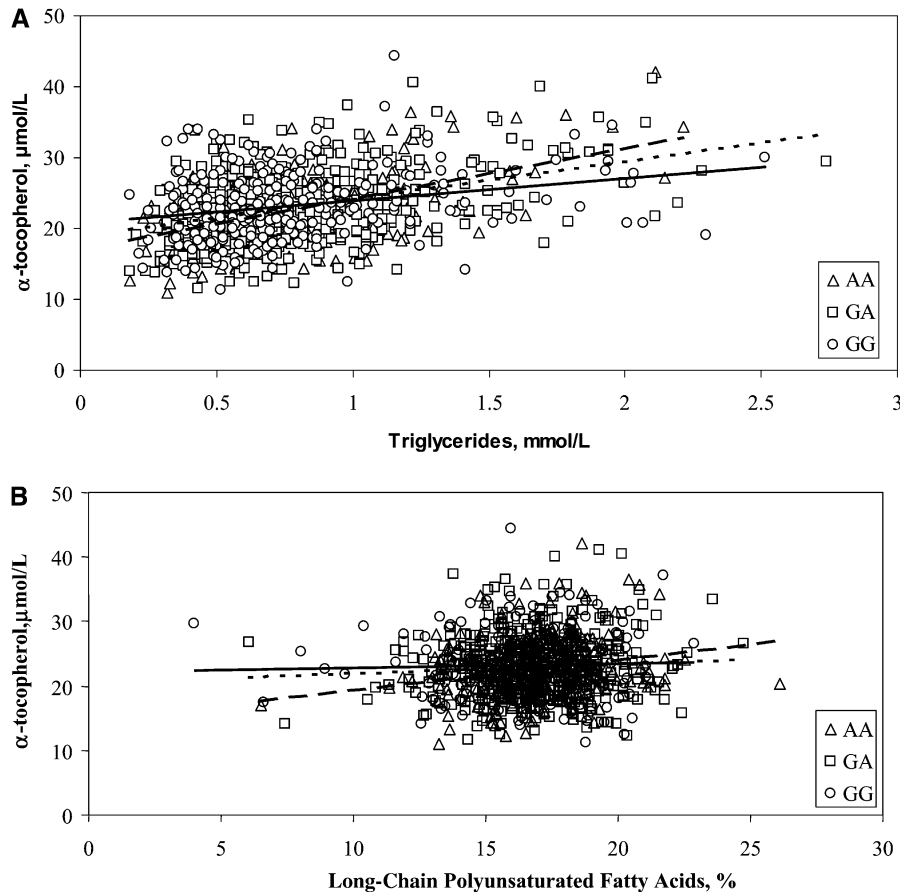
[3] Mean ± SD (all such values).

FIGURE 1. Interactions between the *CD36* rs1527479 single nucleotide polymorphism and plasma α-tocopherol concentrations for (A) fasting serum triglyceride concentrations (*P* for interaction = 0.006) and (B) long-chain polyunsaturated fatty acids (*P* for interaction = 0.005). The regression lines of α-tocopherol compared with triglyceride concentrations were drawn for *GG* (n = 278; ○, continuous line), *GA* (n = 493; □, small-dashed line), and *AA* (n = 222; △, large-dashed line). The regression lines of α-tocopherol compared with long-chain polyunsaturated fatty acid concentrations were drawn for *GG* (n = 259; ○, continuous line), *GA* (n = 450; □, small-dashed line), and *AA* (n = 203; △, large-dashed line).

software, version 8.02; SAS Institute Inc, Cary, NC). All tests were adjusted for age, sex, BMI, and (in the HELENA study only) center. Bonferroni correction was applied to take multiple testing into account (*P* threshold = 0.0071, ie, 0.05/7 independent SNPs). The extent of intercenter heterogeneity or the presence of an interaction with lipid and lipoprotein concentrations was tested by using an interaction term (lipid × SNP) in the GLM.

RESULTS

In the SU.VI.MAX sample, only haplotype analyses could be performed. The haplotype distributions were similar between the whole sample and the subsample (*P* = 0.95). These analyses showed that 2 haplotypes tended to be associated with higher plasma α-tocopherol concentrations (*P* = 0.08 and *P* = 0.09) (**Table 2**). This result prompted us to investigate the effect of the whole known common genetic variability of *CD36* (10 SNPs) on plasma α-tocopherol concentrations in an independent sample composed of 993 European adolescents (the HELENA Study).

The genotype distributions of the 10 *CD36* SNPs in the HELENA Study are presented in Supplementary Table 1 under "Supplemental data" in the online issue. All of the observed SNP genotype frequencies conformed to Hardy-Weinberg pro-

portions (*P* > 0.35) (*see* Supplementary Table 1 under "Supplemental data" in the online issue). The LD of the SNPs was described previously (39). Associations between the 10 *CD36* SNPs and circulating α-tocopherol concentrations were then assessed (**Table 3**). We identified associations between plasma α-tocopherol concentrations and 3 SNPs (rs1984112, rs1761667, and rs1527479). Adolescents who were homozygous for the minor alleles of rs1984112 (*G* allele), rs1761667 (*G* allele), or rs1527479 (*A* allele) had lower plasma α-tocopherol concentrations than did carriers of the respective major alleles: −3.2% (*P* = 0.053), −2.9% (*P* = 0.046), and −3.7% (*P* = 0.0061), respectively. Note that rs1984112 and rs1527479 were in relatively high LD (D′ = 0.95, r^2 = 0.63), whereas rs1761667 and rs1527479 were in almost complete LD (D′ = 1, r^2 = 0.93) and thus reflect the same signal. After correction for multiple testing (*P* threshold < 0.0071), only the association between rs1527479 and α-tocopherol concentrations remained significant.

We also performed haplotype analyses; 10 haplotypes with a frequency ranging from 0.01 to 0.44 accounted for 97% of all combinations. The haplotypic structure of *CD36* could be fully characterized by a subset of 7 HtSNPs: rs1984112, rs1527479 (or rs1761667), rs3211816, rs3211867 (or rs3211883), rs3211908, rs3211931 (or rs1049673), and rs1527483. When the 7 HtSNPs in the haplotype analyses were used, the test for an overall haplotypic
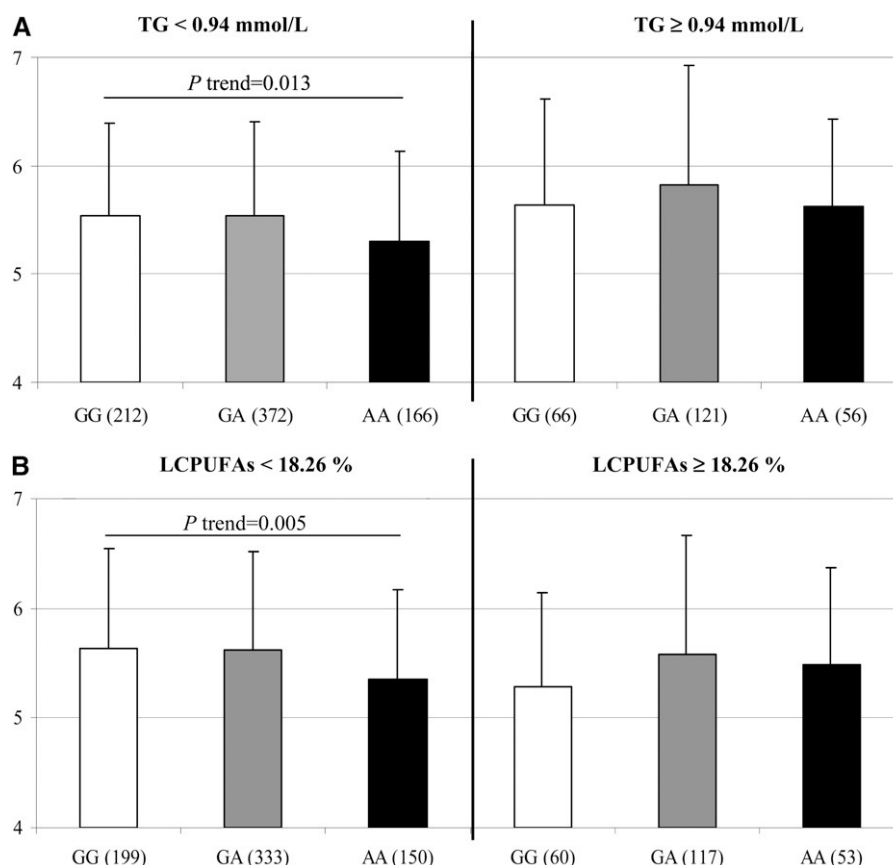
FIGURE 2. Influence of the *CD36* rs1527479 single nucleotide polymorphism on plasma α-tocopherol concentrations in individuals presenting with serum triglyceride (TG) concentrations <0.94 mmol/L (left panel; $n = 750$) or ≥0.94 mmol/L (right panel; $n = 243$) (A) or long-chain polyunsaturated fatty acid (LCPUFA) concentrations <18.26% (left panel; $n = 682$) or ≥18.26% (right panel; $n = 230$) (B). Values are expressed as means ± SD of plasma α-tocopherol/total cholesterol in individuals with the *GG* (white bars), *GA* (gray bars), or *AA* (black bars) genotype. *P* values were calculated for total cholesterol–corrected α-tocopherol values by using a general linear model and were adjusted for age, sex, center, and BMI.

effect on plasma α-tocopherol concentrations was not significant ($P = 0.48$ with 9 df). The most informative and parsimonious haplotype configurations with respect to plasma α-tocopherol concentrations were those including either rs1527479 or rs1984112 alone (*see* Supplementary Table 2 under "Supplemental data" in the online issue), in accordance with the single-locus analyses (Table 3). The rs1527479 SNP accounted for 4.3% of the variance in the α-tocopherol concentration.

Finally, as CD36 mediates the cellular uptake of some lipoproteins and FAs, themselves implicated in vitamin E transport, we searched for potential interactions between the *CD36* rs1527479 SNP, plasma α-tocopherol concentrations, and serum triglyceride, LDL-cholesterol, HDL-cholesterol, saturated FA (SFA), monounsaturated FA (MUFA), PUFA, or LC-PUFA concentrations. Significant interactions were detected with triglyceride ($P = 0.006$; **Figure 1**A) or LC-PUFA ($P = 0.005$) concentrations (Figure 1B) but not with LDL-cholesterol ($P = 0.24$), HDL-cholesterol ($P = 0.61$), SFA ($P = 0.30$), MUFA ($P = 0.60$), or PUFA ($P = 0.26$) concentrations. Note that triglyceride and LC-PUFA concentrations were modestly correlated ($r^2 = 0.14$, $P < 0.0001$). We then stratified the sample on the concentration of serum triglycerides at the 75th percentile (corresponding at 0.94 mmol/L), the cutoff matching closely the crossing of the linear regression lines on Figure 1. We observed that in individuals with triglycerides <0.94 mmol/L, those carrying the A minor allele had lower plasma α-tocopherol concentrations than did those carrying *GG* (P for trend = 0.013; **Figure 2**). In contrast, no association between rs1527479 and plasma α-tocopherol concentrations was found in individuals with a triglyceride concentration ≥0.94 mmol/L (P for trend = 0.70; Figure 2). Similar associations were observed for LC-PUFA concentrations. When stratifying the sample on the concentration of LC-PUFAs at the 75th percentile (corresponding at 18.26%), we observed that in individuals with LC-PUFAs < 18.26%, those carrying the A minor allele had lower plasma α-tocopherol concentrations than *GG* individuals (P for trend = 0.005; Figure 2). In contrast, there was no association between rs1527479 and plasma α-tocopherol concentrations in individuals with LC-PUFAs ≥ 18.26% (P for trend = 0.16; Figure 2). Comparable results were obtained for rs1984112 or rs1761667 (data not shown).

DISCUSSION

To our knowledge, this study was the first to report an association between SNPs in *CD36* and plasma α-tocopherol concentrations. The results of the present association study, together with previous data showing that α-tocopherol is able to modulate CD36 gene expression (40–43), suggest that the scavenger receptor CD36/FAT, best known for its involvement in long-chain FA uptake by adipose tissue (44–47), might also be involved in the metabolism of vitamin E.

The mechanism by which CD36 affects blood vitamin E concentrations is not known. CD36 recognizes a broad variety of lipid ligands, including FAs, oxidized LDL, apoptotic cells, and β-carotene (48). Thus, recognition of vitamin E by CD36 is plausible. In the intestinal lumen, vitamin E is solubilized into mixed micelles (49). One can imagine that, as with SR-BI (50), CD36 may function as a docking port and thus facilitate the transfer of lipid molecules (including tocopherols) from mixed micelles to the apical membrane of the enterocyte. In blood, vitamin E is almost exclusively transported by lipoproteins. Its presence inside cells in a range of different tissues shows that it is being transferred (either directly or indirectly). Although the molecular mechanisms underlying this transport are not known, several hypotheses have been proposed. The first involves uptake of LDL-bound vitamin E by the LDL receptor. The second features transfer of vitamin E from the surface of triglyceride-rich lipoproteins into cells during triglyceride hydrolysis by endothelial lipases. In the third mechanism, HDL-bound vitamin E is taken up by the SR-BI receptor. This latter hypothesis is supported by data from SR-BI–deficient mutant mice, in which α-tocopherol metabolism is abnormal (14). The association between plasma α-tocopherol concentrations and the *CD36* SNPs observed in our study suggests that another mechanism could be involved in vitamin E tissue uptake.

A recent study has shown that some SNPs in *CD36* influenced monocyte and/or platelet CD36 protein expression in African Americans (51). In this work, the authors studied the functionality of 15 SNPs in *CD36* and found that the *A* allele of rs1761667 (as for 3 other SNPs) decreased CD36 expression in monocytes. Because the major *A* allele of rs1761667 is in strong LD ($r^2 = 0.93$) with the major *G* allele of rs1527479 in the HELENA Study, these functional data suggest that the lower plasma α-tocopherol concentrations observed in homozygous carriers of the minor alleles rs1527479 or rs1761667 might be related to a higher CD36 expression and therefore higher FA and vitamin E transport.

We also observed that the concentration of fasting serum triglycerides or LC-PUFAs modulated the association between *CD36* SNPs and plasma α-tocopherol concentrations. The *CD36* rs1527479 *A* allele was associated with lower plasma α-tocopherol concentrations in individuals having serum triglyceride or LC-PUFA concentrations below the 75th percentile only. It is possible that the effect of the *CD36* rs1527479 SNP is more visible when the quantity of triglyceride particles is low than when it is high (therefore transporting more α-tocopherol and saturating CD36). The results with the LC-PUFAs may be related to the fact that the specific function of α-tocopherol is to protect long-chain FAs from oxidation and maintain their concentrations in the membranes adequate for important signaling events (*see* reference 52 for review), especially when the concentration of LC-PUFAs in the membranes is not high (below the 75th percentile).

To date, only one GWAS ($n = 3891$ individuals) has sought to identify the key genes involved in plasma concentrations of carotenoids and tocopherols (26). The authors did not report any associations between *CD36* SNPs and α-tocopherol concentrations. In contrast, they showed that the *APOA5* rs12272004 SNP [in LD with the *APOA5* rs3135506 (S19W) SNP] was associated with α-tocopherol concentrations [allele effect size = +0.072, $P = 7.8 \times 10^{-8}$, or +0.055 ($P = 0.002$) when adjusted for triglyceride concentrations]. Moreover, 2 other SNPs in the *APOA5* locus (rs3135506 and rs662799) known to be associated with triglyceride concentrations were also associated with α-tocopherol concentrations in both this GWAS and a previous study including 169 nonsmoking type 2 diabetic patients (24). In the HELENA Study, we were able to confirm the association between the *APOA5* rs662799 SNP and α-tocopherol concentrations (allele effect size = +0.20 μmol/mmol cholesterol; $P = 0.003$; data not shown). This suggests that our study was sufficiently powered to detect established associations with α-tocopherol concentrations. The fact that Ferruci et al's (26) GWAS did not report an association between *CD36* and the α-tocopherol concentration may signify that CD36 is less involved in the modulation of vitamin E concentrations than is *APOA5*. The need to avoid false-positive associations in a GWAS requires a dramatically reduced P value threshold ($P < 5 \times 10^{-7}$); therefore, weaker associations are not reported.

In conclusion, the present study suggests that CD36/FAT is involved in vitamin E metabolism. Further studies are necessary to identify the cellular mechanisms involved and to establish their importance (relative to other genes/polymorphisms) in modulating blood vitamin E concentrations.

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The American Journal of Clinical Nutrition

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