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Increased concentrations of circulating vitamin E in carriers of the apolipoprotein A5 gene −1131T>C variant and associations with plasma lipids and lipid peroxidation

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Abstract **The aim of this study was to investigate the effects of the apolipoprotein A5 (APOA5) 1131T>C gene variant on vitamin E status and lipid profile. The gene variant was determined in 297 healthy nonsmoking men aged 20–75 years and recruited in the VITAGE Project. Effects of the genotype on vitamin E in plasma, LDL, and buccal mucosa cells (BMC) as well as on cholesterol and triglyceride (TG) concentrations in plasma and apolipoprotein A-I (apoA-I), apoB, apoE, apoC-III, and plasma fatty acids were determined. Plasma malondialdehyde concentrations as a marker of in vivo lipid peroxidation were determined. C allele carriers showed significantly higher TG, VLDL, and LDL in plasma, higher cholesterol in VLDL and intermediate density lipoprotein, and higher plasma fatty acids. Plasma α-tocopherol (but not γ-tocopherol, LDL α- and γ-tocopherol, or BMC total vitamin E) was increased significantly in C allele carriers compared with homozygote T allele carriers ($P = 0.02$), but not after adjustment for cholesterol or TG. Plasma malondialdehyde concentrations did not differ between genotypes. In conclusion, higher plasma lipids in the TC+CC genotype are efficiently protected against lipid peroxidation by higher α-tocopherol concentrations. Lipid-standardized vitamin E should be used to reliably assess vitamin E status in genetic association studies.—** Sundl, I., M. Guardiola, G. Khoschsorur, R. Solà, J. C. Vallvé, G. Godàs, L. Masana, M. Maritschnegg, A. Meinitzer, N. Cardinault, J. M. Roob, E. Rock, B. M. Winklhofer-Roob, and J. Ribalta. **Increased concentrations of circulating vitamin E in carriers of the apolipoprotein A5 gene −1131T>C variant and associations with plasma lipids and lipid peroxidation.** ***J. Lipid Res.*** **2007.** 48: **2506–2513.**

Supplementary key words polymorphism • single nucleotide polymorphism • α-tocopherol • γ-tocopherol • triglycerides • cholesterol • plasma fatty acids • lipoproteins

Apolipoprotein A5 (APOA5) is located near the region of the APOA1/C3/A4 gene cluster on chromosome 11 involved in the regulation of triglyceride (TG) metabolism. The role that APOA5 plays in such regulation has been demonstrated extensively in genetically modified animal models (1–3) and in a large number of association studies (4–10). Mice overexpressing the APOA5 gene have 65% lower plasma TG, whereas the APOA5 knockout mouse develops ~4-fold higher TG concentrations (1). Association studies using different APOA5 markers have clearly shown that apolipoprotein A-V (apoA-V), despite its low plasma concentrations (11), is probably the strongest determinant of circulating TG concentrations (4–10). The most frequently analyzed variant is −1131T>C, and the C allele has been consistently associated with higher TG levels (4–10). The exact function of apoA-V is not known, although in vitro evidence shows that it may control plasma TG by downregulating hepatic VLDL synthesis and stimulating LPL activity (1, 12).

Circulating TG are transported within lipoproteins together with other lipophilic compounds such as vitamin E

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Abbreviations: apoA-V, apolipoprotein A-V; APOA5, apolipoprotein A5; BHT, butylated hydroxytoluene; BMC, buccal mucosa cell; BMI, body mass index; IDL, intermediate density lipoprotein; SFA, saturated fatty acid; TG, triglyceride.

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(13). Consequently, TG concentrations are closely related to vitamin E concentrations, resulting in higher plasma vitamin E concentrations in hyperlipidemic subjects compared with normolipidemic subjects when not corrected for plasma lipids (14).

Vitamin E is an essential fat-soluble nutrient comprising eight different isoforms, including α-, β-, γ-, and δ-tocopherols and their respective tocotrienols, which differ in both number and position of methyl groups on the chroman ring and in having a saturated (tocopherols) or unsaturated (tocotrienols) side chain. Together with dietary lipids, all of these vitamin E isoforms are absorbed equally from the gastrointestinal tract and secreted into chylomicrons. In the liver, the α-tocopherol transfer protein preferentially recognizes RRR-α-tocopherol for incorporation into lipoproteins, whereas the other vitamin E forms are excreted into bile and metabolized and excreted into urine (15, 16). Consequently, α-tocopherol, which also shows the highest biologic activity, is the major form of vitamin E in human plasma and tissue.

The best known function of vitamin E relates to its capacity to scavenge reactive oxygen species, thus acting as a chain-breaking antioxidant inhibiting lipid peroxidation. As an example, α-tocopherol inhibits LDL oxidation initiated by copper ions in an ex vivo model (17). Beside these antioxidant actions, vitamin E also possesses anti-inflammatory functions, such as inhibition of platelet adhesion, inhibition of monocyte proatherogenic activity, and improvement of endothelial functions (18).

Transfer of circulating vitamin E from lipoproteins into tissue is regulated by mechanisms that also control lipid metabolism. The enzyme LPL is able to transfer tocopherols during TG hydrolysis to extrahepatic tissues (19), whereas the phospholipid transfer protein exchanges α-tocopherol between different lipoprotein classes (20). Furthermore, vitamin E transported in LDL is delivered to cells via a receptor-mediated uptake of LDL (21). Consequently, lipid, lipoprotein, and vitamin E concentrations correlate strongly with each other, indicating that their metabolic pathways are tightly linked. Therefore, it is conceivable that environmental and genetic factors affecting lipid metabolism would affect vitamin E status.

The hypothesis of the present study was that the APOA5 genotype not only influences lipid metabolism but, as a result of the mechanisms described above, also alters vitamin E metabolism in humans. To test this hypothesis, we investigated the effects of the APOA5 variant −1131T>C on lipid status in a population of 297 healthy male nonsmoking subjects aged 20–75 years and whether these changes have repercussion on vitamin E status. A detailed lipoprotein profile, including total lipids and apolipoproteins plus the lipid and apolipoprotein contents of all lipoprotein fractions, was determined along with vitamin E concentrations in plasma, LDL, and buccal mucosa cells (BMC). In addition, we studied the effects of the APOA5 variant-associated differences in vitamin E and lipid status on malondialdehyde, a biomarker of in vivo lipid peroxidation, which has been used successfully in patients with increased plasma lipids (22).

METHODS

Study subjects

In this cross-sectional study, 299 healthy male nonsmoking subjects (0 cigarettes/day for >6 months), aged 20–75 years (stratified by age), were recruited in Clermont-Ferrand, France (n = 99), Graz, Austria (n = 100), and Reus, Spain (n = 100) as part of the European Commission-funded research and technology development project of the 5th Framework Program, specific research and technology development Program Quality of Life and Management of Living Resources, Key Action 1, Food, Nutrition, and Health, entitled Vitamin A, Vitamin E, and Carotenoid Status and Metabolism during Ageing: Functional and Nutritional Consequences, acronym VITAGE (Contract QLK1-CT-1999-00830) (23). After informative sessions, a trained medical doctor conducted a personal interview to obtain information on anthropometric measurements, personal history, lifestyle, use of medications, physical activity, smoking habits, and use of dietary supplements containing vitamins or trace elements. Exclusion criteria were familial hypercholesterolemia, chronic diseases (including diabetes, cancer, cardiac insufficiency, neurological diseases, inflammatory diseases and chronic diseases of the liver, lung, or thyroid, nonstable hypertension, dementia, and infectious diseases known to affect the immune system, such as human immunodeficiency virus and hepatitis C), vaccination during the past 2 months, alcoholism or drug addiction, competitive sports activities, and the consumption of special diets or dietary supplements in the past 3 months. The study protocol was approved by the Ethics Committees of the three recruiting centers, and written informed consent was obtained from all participants.

Sample collection

Collection of blood samples and preparation of plasma and buffy coats. After an overnight fast, venous blood was drawn into plastic tubes containing EDTA to obtain EDTA plasma (Sarstedt, Ltd., Nümbrecht, Germany), protected from light, and centrifuged immediately at 1,500 *g* at 8°C for 10 min. Plasma was separated and divided into aliquots. For LDL isolation, a 60% sucrose solution was added to plasma to obtain a final concentration of 0.6% (24). For genomic DNA isolation, buffy coat was collected from blood drawn on EDTA. All samples were stored at −80°C until determination of analytical variables.

Collection of BMC. After rinsing the mouth with drinking water, BMC were collected as described by Gilbert et al. (25). Briefly, study subjects were asked to brush the inside of their cheeks with a soft toothbrush 20 times on one side and rinse the mouth with 25 ml of isotonic table salt solution (0.9% sodium chloride) and then repeat the procedure on the other side. The two rinsing volumes were collected in a single tube and centrifuged at 1,400 *g* for 10 min at 4°C. The supernatant was discarded, and the cell pellet was washed with 15 ml of cold phosphate-buffered saline solution. After vortexing, the sample was centrifuged as above and the supernatant was removed. The cell pellet was resuspended in 1.2 ml of cold PBS, flushed with nitrogen, and stored at −80°C until determination of vitamin E.

Five day food records. For dietary assessment, subjects recorded all food items and drinks consumed during a 5 day period. On the basis of these food records, the intake of total fat, saturated fatty acids (SFA), MUFA, and PUFA was calculated. For calculation of nutrients, the REGAL food composition tables (26, 27) and the software package of the Austrian food composition table Ernährungswissenschaftliches Programm (dato Denkwerkzeuge, Vienna, 1997) were used.

Determination of plasma lipids

Determination of TG and cholesterol. TG and cholesterol concentrations in plasma and lipoprotein fractions were measured using enzymatic kits (F. Hoffmann-La Roche, Ltd.) adapted for a Cobas Mira centrifugal analyzer (F. Hoffmann-La Roche) with Precilip EL® and Precinorm® (F. Hoffmann-La Roche) as quality controls. Immunoturbidometry was used for measurement of the apolipoproteins using specific antiserum purchased from F. Hoffmann-La Roche (for apoA-I and apoB), Daiichi Chemicals (for apoE and apoC-III), and Incstar Corp. (for lipoprotein [a]).

Sequential preparative ultracentrifugation. Lipoproteins were separated by sequential preparative ultracentrifugation using a Kontron 45.6 fixed-angle rotor in a Centrikon 75 (Kontron Instruments). The following lipoprotein fractions were isolated: VLDL ($d < 1.006$ g/ml), intermediate density lipoprotein (IDL; $d = 1.006$–1.019 g/ml), and LDL ($d = 1.019$–1.063 g/ml). Total HDL cholesterol was measured subsequent to the precipitation of the apoB-containing lipoproteins with polyethylene glycol (Immuno AG).

Determination of plasma total fatty acids. The determination of the fatty acid content in plasma was based on an esterification procedure and a subsequent GC analysis of the fatty acid methyl esters as described by Sattler et al. (28). Briefly, 450 μl of EDTA plasma and 100 μl of internal standard (10 mg of heptadecanoic acid and 100 mg of butylated hydroxytoluene (BHT) in 10 ml of methanol) were added to a Teflon screw-capped tube, vortexed for 10 s, and then kept at −80°C for a minimum of 30 min. The deep-frozen suspension was freeze-dried on the lyophilisator (Virtis; Servo Laboratories, Graz, Austria) for ~15 h. Thereafter, 1 ml of boron trifluoride-methanol complex and 500 μl of toluene were added and vortexed for 20 s Transesterification was performed at 110°C for 90 min. After the transesterification step, 2 ml of purified water was added and the fatty acid methyl esters were extracted three times with *n*-hexane. The hexane extracts were dried in a Speed-Vac (Bachhofer; Servo Laboratories) at room temperature for 30 min, redissolved in 450 μl of dichloromethane, and subjected to GC analysis (Hewlett-Packard 5890 Series II; Agilent Technologies, Vienna, Austria). Separation of fatty acid methyl esters was achieved on a DB-23 column (Agilent Technologies) with a length of 30 m and a diameter of 0.250 mm. The mobile phase was a mixture of helium and hydrogen gas. The oven temperature at injection was 150°C and was increased to 222°C (3°C/min) and kept at this temperature for 3 min, then increased further to 238°C (3°C/min) and kept at this temperature for 3 min, and finally increased to 255°C (20°C/min). The areas under the GC peaks were quantified by integration, and the internal standard described above was used for calculation of the amounts of fatty acids. Nineteen plasma samples of the patients were processed along with a control sample from the plasma pool for long-term quality control. Coefficients of variation for the different fatty acids were between 0.38% and 8.3% within run and between 1.7% and 8.6% between run.

Determination of vitamin E

LDL isolation. LDL was isolated as described by Bergmann et al. (24). Briefly, 1.5264 g of solid potassium bromide was added to 4 ml of defrosted EDTA plasma, which had been mixed with sucrose as described above. Separation of LDL was achieved by single-step discontinuous gradient ultracentrifugation in a Beckman NVT65 rotor (Beckman Coulter, Servo Laboratories) at 60,000 rpm for 2 h at 10°C (29). Thereafter, the LDL band was isolated and filtered through a 0.20 μm sterile filter (Corning, Inc., Corning, NY) into an evacuated glass vial (BD Vacutainer; Belliver Industrial Estate, Plymouth, UK) and processed on the same day. LDL density was determined using an Anton Paar DMA 48 density meter (A. Paar, Ltd., Graz, Austria). The cholesterol content of LDL was determined using a kit from Roche Diagnostics Ltd. (F. Hoffmann-La Roche).

Determination of α- and γ-tocopherols in plasma and in LDL. The determination of tocopherols in plasma and in LDL was performed as described by Aebischer, Schierle, and Schuep (30). Briefly, EDTA plasma or isolated LDL was diluted with deionized distilled water and deproteinized with 400 μl of absolute ethanol. To extract lipophilic compounds, 800 μl of *n*-hexane/BHT (350 mg of BHT in 1,000 ml of *n*-hexane) was added and centrifuged, and the clear supernatant was transferred by a dispenser/dilutor system (Micro Lab 500B Dilutor; Hamilton, Martinsried, Germany) to an Eppendorf tube to be dried on a Speed-Vac (Savant, New York, NY). The residue was then redissolved in a mixture of methanol and 1,4-dioxane (1:1), diluted with acetonitrile, and injected into the HPLC system (Hewlett-Packard 1100A; Agilent). Separation was achieved on a reverse-phase column; the mobile phase was a mixture of acetonitrile, tetrahydrofuran, methanol, 1% ammonium acetate solution, and 10 mg of L(+)-ascorbate; the flow rate was 1.6 ml/min. Vitamin E was detected using a fluorescence detector (Jasco model FP-920; Biolab, Vienna, Austria) at extinction of 298 nm and emission of 328 nm (α- and γ-tocopherol). The areas under the HPLC peaks were quantified on an HP Chemstation (Hewlett-Packard 35900E; Agilent). The coefficients of variation within run were 1.26% for α-tocopherol and 0.80% for γ-tocopherol in plasma and 0.70% for α-tocopherol and 1.4% for γ-tocopherol in LDL. The coefficients of variation between day were 1.81% for α-tocopherol and 2.78% for γ-tocopherol in plasma and 2.54% for α-tocopherol and 2.98% for γ-tocopherol in LDL. Six plasma and LDL samples of the subjects were processed along with two control samples from the plasma and LDL pools, respectively, obtained from a number of healthy subjects to be used for long-term quality control along with a standard solution. The detection limit was 0.012 μmol/l for α- and γ-tocopherol. The tocopherol content of LDL was expressed as moles of tocopherol per mole of LDL.

Determination of total vitamin E in BMC. Vitamin E was extracted from a resuspension of BMC in PBS (1 ml) as described by Borel et al. (31). Briefly, after a short sonication (15 s; Labsonic U; B. Braun), α-tocopheryl acetate (Fluka, l'Isle d'Abeau, France) was added to samples as an internal standard. The proteins were precipitated with ethanol precipitation. Then, the vitamin E was extracted twice with hexane. The extract was evaporated to dryness under nitrogen, dissolved in methanol-dichloromethane (65:35, v/v), injected into a C18 column (5 μm, 250 mm × 4.6 mm; Nucléosil; Interchim, Montluçon, France), and assayed by reverse-phase HPLC (HPLC apparatus: Waters 996 UV-vis DAD; Waters SA, St-Quentin-en-Yvelines, France). Pure methanol, at a flow-rate of 2 ml/min, eluted α-tocopherol in 5.0 min and tocopheryl acetate in 6.3 min. The compounds were detected by ultraviolet light (292 nm), then quantified by internal and external calibration using daily-controlled standard solutions. Vitamin E concentrations were standardized for protein, as determined by Lowry et al. (32).

Determination of malondialdehyde in plasma

Plasma malondialdehyde concentrations were determined by HPLC as a biomarker of in vivo lipid peroxidation. The method described by Khoschsorur et al. (33) was used.

APOA5 genotyping

According to the nomenclature and methodology used by Pennacchio and colleagues (1), the single nucleotide polymorphism −1131T>C was used as the genetic marker. Genotyping was performed with primers AV-1 (5′-GATTGATTCAAGATGCATT-TAGGAC-3′) and AV-2 (5′-CCCCAGGAACTGGAGCGAAATT-3′), which forced a *Mse*I (New England Biolabs, Beverly, MA) site for enzymatic restriction.

Statistical methods

Statistical analyses were carried out with SPSS version 14.0. The Chi-square test was used to test for Hardy-Weinberg equilibrium. Normal distribution of data was checked with the Kolmogorov-Smirnov test. Comparisons of age and body mass index (BMI) were made with Student's *t*-test. ANOVA was performed to compare the mean values of lipid, lipoprotein, apolipoprotein, and vitamin E data adjusted for age and BMI, because of well-known associations between plasma lipids, age, and BMI. Linear regression analysis was performed between plasma and LDL vitamin E concentrations. Log-transformation was performed when variables were not normally distributed. Results are expressed as means ± SD or box-and-whisker plots. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic data of the study population

We studied a total of 297 nonsmoking healthy males aged 20–75 years from France, Austria, and Spain. Genetic material was missing for two subjects; thus, two subjects had to be excluded from statistical analysis. There were no differences in mean age, age distribution, and BMI among countries (**Table 1**); therefore, all subjects were pooled for genetic analyses of lipid and vitamin concentrations.

Frequencies of the −1131T>C polymorphism in the APOA5 gene

Among the 297 subjects, 251 had the common genotype (T/T), 45 were heterozygote (T/C), and 1 subject was homozygote (C/C). For association analyses, all carriers of one or two copies of the C allele were pooled. The C allele was carried by 15.5% of the subjects, resulting in an allele frequency of 0.08, which was similar to that found in the Caucasian general population (6). The observed frequencies of the −1131T>C genotypes were not different from those predicted by the Hardy-Weinberg distribution. There were no differences in the allele frequencies between Spain, France, and Austria (Table 1).

Characteristics of the study subjects according to APOA5 genotype

There were no differences in age between homozygote carriers of the T allele (45.8 ± 15.1 years) and carriers of the C allele (48.0 ± 17.1 years). BMI did not differ between the TT (25.0 ± 2.66 kg/m^2) and the TC+CC (24.7 ± 2.65 kg/m^2) genotypes.

Effects of the APOA5 genotype on lipoprotein profile

Carriers of the C allele had 15.2% higher plasma TG concentrations ($P = 0.01$) attributable to increases in VLDL-TG ($P = 0.001$), whereas IDL-TG, LDL-TG, and HDL-TG did not differ significantly between the genotypes (**Table 2**). This was accompanied by a 10% increase in the lipoprotein lipase inhibitor apoC-III in the TC+CC genotype (17.0 ± 3.27 mg/dl) compared with the TT genotype (15.4 ± 3.30 mg/dl) ($P = 0.002$). Total cholesterol concentrations were not different between carriers and noncarriers of the C allele. However, carriers of the C allele showed significantly higher cholesterol concentrations in the VLDL ($P = 0.002$) and IDL ($P < 0.05$) fractions, whereas there were no differences in the LDL and HDL fractions (Table 2). Circulating apoB-containing lipoproteins were also 11% higher in carriers of the C allele (apoB-100; $P = 0.006$) as a result of an accumulation of VLDL and LDL particles, as assessed by their apoB content (VLDL, $P = 0.008$; LDL, $P = 0.03$). In contrast, the APOA5 genotype did not have an effect on HDL (Table 2). There was no difference between the TT and TC+CC genotypes in plasma lipoprotein [a] (23.5 ± 21.7 vs. 23.1 ± 20.0 mg/dl) and apoE (3.50 ± 0.872 vs. 3.48 ± 0.691 mg/dl).

Effects of dietary intake and of the APOA5 genotype on plasma fatty acids

There were no differences in total fat intake or in intake of SFA, MUFA, and PUFA between the genotypes. After dichotomizing dietary PUFA intake according to the study population mean (16.2 ± 6.95 g/day), there was no effect of high or low PUFA intake on TG concentrations, plasma VLDL, and VLDL-TG. Total plasma fatty acids were 8.6% higher in carriers of the C allele compared with homozygote carriers of the T allele (10.4 ± 2.31 vs. 11.3 ± 2.32 mmol/l) ($P = 0.007$). The distribution of fatty acids

TABLE 1. Characteristics of the study population

Characteristics	France (n = 97)	Austria (n = 100)	Spain (n = 100)	Total (n = 297)	P^a
Anthropometric variables					
Age (years)	46.6 ± 15.1	45.5 ± 15.1	46.3 ± 16.0	46.1 ± 15.4	NS
BMI (kg/m^2)	24.6 ± 2.58	25.2 ± 2.71	25.1 ± 2.66	25.0 ± 2.66	NS
Genotype					
TT (%)	84.5	86.0	83.0	84.5	NS^b
TC/CC (%)	15.5	14.0	17.0	15.5	NS^b
C allele frequency	0.08	0.07	0.09	0.08	NS^b

BMI, body mass index. Anthropometric variable values are shown as means ± SD.
[a] France versus Austria versus Spain.
[b] By Chi-square test.

TABLE 2. Lipoprotein profiles according to APOA5 genotype

Lipoprotein	Cholesterol			TG			ApoB/ApoA-I Content		
	TT (n = 251)	TC + CC (n = 46)	P^a	TT (n = 251)	TC + CC (n = 46)	P^a	TT (n = 251)	TC + CC (n = 46)	P^a
	mmol/l			*mmol/l*			*mg/dl*		
Plasma	4.82 ± 0.932	5.06 ± 0.878	NS[d]	1.05 ± 0.522	1.21 ± 0.582	0.01[d,e]	70.6 ± 17.2[b]	78.2 ± 17.4[b]	0.006[d]
							133.5 ± 18.9[c]	135.9 ± 17.89[c]	NS[d]
VLDL	0.298 ± 0.248	0.397 ± 0.290	0.002[d,e]	0.568 ± 0.419	0.751 ± 0.505	0.001[d,e]	3.16 ± 2.18[b]	4.15 ± 2.58[b]	0.008[d,e]
IDL	0.193 ± 0.106	0.224 ± 0.113	0.048[d,e]	0.093 ± 0.337	0.105 ± 0.442	NS[d,e]	2.73 ± 1.37[b]	3.11 ± 1.57[b]	NS[d,e]
LDL	2.68 ± 0.728	2.89 ± 0.652	NS[d]	0.237 ± 1.00	0.188 ± 0.048	NS[d,e]	54.4 ± 14.6[b]	59.7 ± 13.4[b]	0.03[d]
HDL	1.33 ± 0.308	1.33 ± 0.296	NS[d]	0.094 ± 0.031	0.103 ± 0.041	NS[d,e]	112.6 ± 17.4[c]	114.8 ± 16.6[c]	NS[d]

APOA5, apolipoprotein A5; IDL, intermediate density lipoprotein; TG, triglyceride. Values shown are means ± SD.
[a] ANOVA.
[b] ApoB.
[c] ApoA-I.
[d] *P* value standardized for BMI and age.
[e] On log-transformed data.

among SFA, MUFA, and n-3 or n-6 PUFA expressed as mol% did not differ between the genotypes.

In vivo lipid peroxidation according to APOA5 genotype

Plasma malondialdehyde concentrations did not show a significant difference between the TT and TC+CC genotypes, either when the concentrations (TT, 0.68 ± 0.34 μmol/l vs. TC+CC, 0.75 ± 0.38 μmol/l) or when the ratios of malondialdehyde to cholesterol (TT, 0.144 ± 0.074 μmol/mmol vs. TC+CC, 0.155 ± 0.086 μmol/mmol) were used.

Effects of the APOA5 gene on vitamin E status

Carriers of the C allele had significantly higher (9.1%) plasma α-tocopherol concentrations compared with noncarriers ($P = 0.02$) (**Fig. 1**). Because there was a significant relation between plasma α-tocopherol concentrations and TG concentrations ($r = 0.50$, $P < 0.001$) and cholesterol concentrations ($r = 0.75$, $P < 0.001$), standardization was performed. The difference in α-tocopherol concentrations between the genotypes was not statistically significant when standardized for TG and cholesterol (both separately and in combination), nor when measured in the LDL fraction. γ-Tocopherol concentrations as well as total vitamin E concentrations measured in BMC did not differ significantly between carriers and noncarriers of the C allele (**Table 3**). There was a close relation between plasma and LDL α- and γ-tocopherol concentrations in both genotypes (α-tocopherol, $r = 0.45$, $P < 0.001$ in T/T, $r = 0.64$, $P < 0.001$ in C/T and CC; γ-tocopherol, $r = 0.93$, $P < 0.001$ in T/T, $r = 0.94$, $P < 0.001$ in C/T and CC).

DISCUSSION

The hypothesis of this study was that, through its action on lipid metabolism, the APOA5 gene would have an effect on the metabolism and distribution of the fat-soluble vitamin E and thus could alter the status of this most potent lipophilic antioxidant. In the study subjects (i.e., healthy male nonsmoking volunteers), the APOA5 variant −1131T>C had a significant impact on plasma vitamin E concentrations, such that the carriers of the C allele exhibited high α-tocopherol but not γ-tocopherol concentrations in association with increased TG concentrations compared with carriers of the T allele. TG concentrations are well known to be a major determinant of circulating vitamin E concentrations (14); therefore, standardization

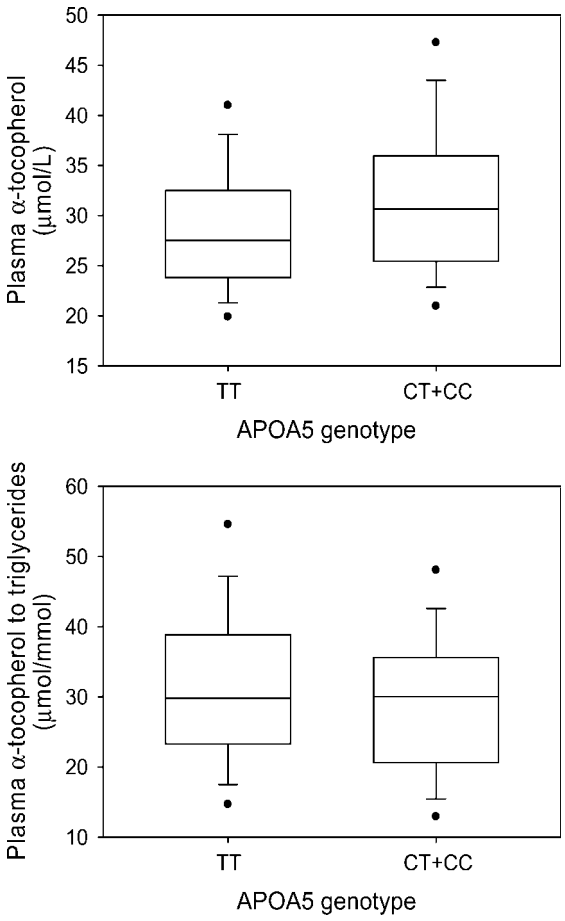

Fig. 1. Differences in plasma α-tocopherol concentrations ($P = 0.02$; upper panel) and α-tocopherol standardized for triglyceride concentrations (not significant; lower panel) between apolipoprotein A5 (APOA5) genotypes. Results are expressed as box-and-whisker plots.

TABLE 3. Vitamin E profile according to APOA5 genotype

Vitamin E Status	APOA5 Genotype		
	TT (n = 251)	TC + CC (n = 46)	P^a
Plasma α-tocopherol (μmol/l)	28.7 ± 6.34	31.3 ± 7.43	0.02[b,c]
Adjusted for cholesterol			NS[b,c]
Adjusted for TG			NS[b,c]
Adjusted for cholesterol and TG			NS[b,c]
Adjusted for apoB			NS[b,c]
Plasma α-tocopherol to cholesterol (μmol/mmol)	5.96 ± 0.907	6.15 ± 1.03	NS[b,c]
Plasma γ-tocopherol (μmol/l)	1.38 ± 0.835	1.46 ± 0.689	NS[b,c]
Plasma γ-tocopherol to cholesterol (μmol/mmol)	0.285 ± 0.154	0.290 ± 0.126	NS[b,c]
LDL α-tocopherol (μmol/l)	8.28 ± 1.10	8.51 ± 1.25	NS[b]
LDL γ-tocopherol (μmol/l)	0.329 ± 0.180	0.329 ± 0.138	NS[b,c]
Buccal mucosa cell total vitamin E (ng/mg protein)	119.0 ± 95.8	110.0 ± 55.6	NS[b,c]

Values shown are means ± SD.
[a] ANOVA.
[b] *P* value standardized for BMI and age.
[c] On log-transformed data.

for TG concentrations was performed. After standardization, the effect of the genotype disappeared, indicating that increased vitamin E concentrations are a function of increased TG concentrations. This relationship is further supported by the highly significant linear regression of α-tocopherol concentrations on TG concentrations in the present study.

APOA5 and vitamin E

The reason for this differential effect of the −1131T>C variant of the APOA5 gene on plasma α-tocopherol compared with γ-tocopherol concentrations may relate to the fact that, although intestinal absorption of the different vitamin E isoforms is similar, α-tocopherol transfer protein in the liver preferentially binds the α-tocopherol, which is then incorporated into nascent VLDL particles (15, 16). Although this metabolic pathway is responsible for the enrichment of α-tocopherol in lipoproteins and, consequently, also in tissues, γ-tocopherol is either excreted via bile or metabolized in the liver to be excreted as the water-soluble γ-carboxyethyl-hydroxychroman in the urine (15, 16). Because in vitro data suggest that apoA-V may influence hepatic VLDL synthesis (34), the incorporation of α-tocopherol into VLDL could be modulated by the −1131T>C genotype. This results not only in higher TG concentrations in the carriers of the C allele but also in higher α-tocopherol concentrations. In contrast, because of the lack of incorporation of γ-tocopherol into nascent VLDL, γ-tocopherol concentrations are not affected by the genotype. This could explain the absence of genotype-related differences in both plasma and LDL γ-tocopherol concentrations observed in the present study.

The extrahepatic tissue uptake and distribution of vitamin E is directed mainly by LPL (19). Because apoA-V stimulates LPL activity (2, 35), the APOA5 genotype could also influence the distribution of vitamin E between plasma and tissues. Our results indicate that increased α-tocopherol concentrations are entirely attributable to increased plasma lipid concentrations. Given that there was no difference in LDL-TG content between the genotypes, it is not surprising that there was also no difference in LDL α-tocopherol concentrations. Because micronutrient concentrations in BMC have been suggested to reflect vitamin E status better than plasma concentrations (36), we determined vitamin E in BMC. There were no differences between the APOA5 genotypes in BMC vitamin E, in agreement with LDL vitamin E concentrations and vitamin E plasma concentrations standardized for lipid concentrations.

APOA5 and lipids and lipoproteins

The crucial role of apoA-V in TG metabolism has been demonstrated in several transgenic and knockout animal models (1–3) as well as in epidemiological studies investigating single nucleotide polymorphisms at the human APOA5 locus (5, 9, 10). In the present study, we aimed to study its effect on a population of subjects of different ages specifically recruited to qualify as healthy and with a complete lipid and lipoprotein profile. Carriers of the C allele had increased TG concentrations compared with wild-type subjects, which were mainly attributable to increases of TG in the VLDL fraction. Carriers of the C allele also had higher VLDL and IDL cholesterol and higher apoB concentrations, indicating a significantly higher number of apoB-containing lipoproteins. Similar observations have been made previously (2, 37). However, it remains to be determined whether increased synthesis, decreased clearance of TG-rich lipoproteins, or both are the underlying mechanisms of the effects of apoA-V on lipid status. Our results support both possibilities, because carriers of the C allele had increased plasma concentrations of free fatty acids and apoC-III. Although increased concentrations of free fatty acids are known to induce hepatic synthesis of VLDL, increased concentrations of apoC-III can impair the ability to hydrolyze TG as well as the removal of lipoprotein remnants. This could explain all of the observed features associated with the −1131T>C gene variant, namely, increased VLDL, increased TG, and increased IDL (VLDL remnants).

Increased plasma fatty acid concentrations did not show a specific pattern of increased fatty acids. Recently, a modulating effect of dietary n-6 PUFA on plasma lipids in carriers of the C allele was shown (38). However, in the present study, such an effect was not observed.

This study was not designed to test the hypothesis that differences in vitamin E status attributable to the APOA5 variant −1131T>C would affect biomarkers of oxidative stress and inflammation. Only healthy volunteers were enrolled who had passed strict inclusion/exclusion criteria, such that they did not have increased markers of inflammation such as C-reactive protein, leukocyte count, or bands. At the same time, they had to be nonsmokers, with BMI < 30 kg/m^2, and free of acute or chronic diseases, making an increased oxidative stress status very unlikely. They were also in a fasted state, such that postprandial lipid peroxidation would not be investigated. Furthermore, plasma α-tocopherol standardized for lipids [i.e., α-tocopherol to cholesterol of 5.96 ± 0.91 μmol/l (TT) compared with 6.15 ± 1.03 μmol/l (TC+CC)] was not associated with significant differences in lipid peroxidation, because higher lipids in the TC+CC genotype are associated with higher α-tocopherol concentrations, resulting in comparable protection against lipid peroxidation.

In summary, the data presented here indicate that the APOA5 gene significantly alters the lipoprotein profile even in healthy subjects. Such an effect is accompanied by increased circulating vitamin E concentrations as a result of increased TG concentrations, whereas vitamin E metabolism does not seem to be affected. From a health perspective, the association of increased plasma lipids with an increase in α-tocopherol concentrations is highly relevant for ensuring efficient protection against lipid peroxidation. These results should be taken into account when interpreting plasma vitamin E concentrations in humans. Given the fact that the −1131T>C variant affects lipid status and, as a consequence, also alters plasma vitamin E concentrations, this study further supports the use of vitamin E standardized for lipids to reliably assess vitamin E status, particularly in genetic association studies.

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