Influence of apolipoprotein E genotype on fat-soluble plasma antioxidants in Spanish children

Henar Ortega, Patricia Castilla, Diego Gómez-Coronado, Carmen Garcés, Mercedes Benavente, Fernando Rodríguez-Artalejo, Manuel de Oya, and Miguel A Lasunción

ABSTRACT

Background: Apolipoprotein (apo) E is a major determinant of plasma lipid concentrations, which in turn influence the plasma concentrations of various fat-soluble vitamins.

Objective: We aimed to analyze the effect of APOE genotype on fat-soluble antioxidant concentrations in children.

Design: A total of 926 healthy boys and girls aged 6–8 y were selected from 4 cities in Spain. APOE genotyping was carried out, and plasma concentrations of lipids, apolipoproteins, and lipid-soluble antioxidants were measured.

Results: Plasma lipid concentrations were strongly influenced by APOE genotype. The mean plasma concentration of α-tocopherol was 21.3 μmol/L, which is one of the highest values ever reported for a population of children. Although plasma concentrations of α-tocopherol, γ-tocopherol, lycopene, and α-carotene varied significantly between subjects with different APOE genotypes, most of these differences disappeared after adjustment for lipoprotein-related covariates. Nevertheless, tocopherol concentrations remained elevated in individuals with the E2/2 genotype. Multivariate regression analysis showed interactions of APOE genotype with triacylglycerol and apo B in determining α-tocopherol concentrations. When subjects were stratified according to major apo E groups, apo B appeared to be the most important predictor of α-tocopherol concentrations in all groups, whereas triacylglycerol was identified only in carriers of the E2 allele.

Conclusions: The association between APOE genotype and lipophilic antioxidant concentrations is dependent mainly on the effect of the polymorphism on lipoprotein concentrations. However, triacylglycerol plays a role in determining the variability of α-tocopherol concentrations in E2 carriers only. This suggests that the α-tocopherol content in each lipoprotein class varies according to APOE genotype. Am J Clin Nutr 2005;81:624–32.

KEY WORDS Apolipoprotein E, tocopherols, carotenoids, antioxidants, lipoproteins, children

INTRODUCTION

Oxidative stress is believed to underlie most prevalent chronic diseases in industrialized countries, such as cancer, atherosclerosis, and neurodegenerative diseases (1–3). Although morbidity occurs mainly at advanced ages, nutritional factors operating in childhood, which possibly interact with genetic factors, may also contribute to the development of such diseases.

In plasma, lipoprotein-bound tocopherols and carotenoids protect fatty acids from peroxidation, thereby preserving their function and the normal fate of lipoprotein particles. Plasma concentrations of vitamin E and carotenoids are correlated with those of plasma lipids (4), which in turn are affected by genetic factors, such as the polymorphism associated with the APOE gene. Apolipoprotein E (apo E) modulates the hepatic binding, uptake, and catabolism of several classes of lipoproteins (5, 6). Three major isoforms, referred to as apo E2, E3, and E4 and which are encoded by alleles ε2, ε3, and ε4 respectively, have been identified, but their frequencies vary between populations. In particular, the frequency of ε4 in northern Europe and North America is significantly higher than in Mediterranean or Asian countries (5, 7–9). Possession of the apo E4 form increases both total and LDL-cholesterol concentrations over those seen with apo E3, whereas apo E2 has the opposite effect (5, 10). This effect of the apo E polymorphism may be modulated by other factors, such as sex (9–11) and alcohol intake (12). Taking ε3 allele homozygosity as a reference, we found that in children, the presence of the ε2 allele lowers LDL-cholesterol and apo B plasma concentrations by 18% and 19%, respectively, whereas the presence of the ε4 allele significantly increases these variables by 7% and 8%, respectively (13).

In addition to modulation of lipoprotein metabolism, apo E appears to have other functions in the organism (6, 14). In particular, it protects against lipid peroxidation; apo E2 exhibits the highest antioxidant activity, followed by apo E3 and then apo E4 (15, 16). This may be relevant to the association between the ε4

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allele and the risk of Alzheimer disease (17). In this context, it has been suggested that APOE genotyping may have medical applications in the identification of patient subgroups susceptible to oxidative damage who could then be treated with antioxidant vitamins (18).

In adult subjects, lipid-adjusted vitamin E plasma concentrations appear not to differ between the major apo E phenotypes (19). However, an association of vitamin A with the apo E polymorphism was found in women, with apo E2 carriers exhibiting a slightly higher concentration than other subjects (19). These results indicate an influence of the apo E polymorphism on antioxidant vitamins, which may be modulated by sex (19).

In the present work, we analyze the influence of APOE genotype on plasma concentrations of tocopherols, carotenoids, and retinol in a large prepubertal infant population free of an established hormonal influence. This is also the first survey of fat-soluble antioxidant status in Spanish children.

SUBJECTS AND METHODS

Subjects

This work forms part of the Four Provinces Study, a large-scale study designed to analyze cardiovascular disease risk factors in Spain by studying children from 4 large metropolitan areas of the country. In the present study, a total of 926 healthy children, 441 boys and 485 girls, aged 6–8 y (boys, 6.66 ± 0.64 y; girls, 6.70 ± 0.63 y; \( \bar{x} \pm SD; P > 0.05 \)) were studied. All the subjects were free of ischemic heart disease and any other endocrine, metabolic, hepatic, or renal disorder. The study protocol was approved by the Clinical Investigation Ethics Committee of the Fundación Jiménez Díaz. The work is consistent with the principles contained within the Declaration of Helsinki and subsequent reviews, as well as the prevailing Spanish legislation on clinical research in human subjects. Parents were required to sign a written consent for their child’s inclusion in the study. A team consisting of one physician and several nurses (who were responsible for blood extractions and physical measurements) was in charge of the fieldwork. Details of sample selection are published elsewhere (13).

Methods

Weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Body mass index (BMI; weight in kilograms divided by height in meters squared, ie, kg/m^2) was calculated from these parameters. No statistically significant differences in BMI were found between the boys and the girls (boys, calculated from these parameters. No statistically significant differences in BMI were found between the boys and the girls (boys, 16.9 ± 2.4; girls, 17.0 ± 2.6; \( \bar{x} \pm SD; P > 0.05 \)). The percentage of subjects classified as overweight (BMI > 20.1) was 11.5% in boys and 12.6% in girls (\( P > 0.05 \)).

The children were asked to fast overnight. Venous blood samples were then obtained early in the morning by venipuncture into evacuated tubes containing EDTA-Na2 as an anticoagulant, placed on ice, and centrifuged immediately at 1500 \( \times g \), 4 °C, for 25 min. The plasma was separated and used for biochemical determinations, and the cells were kept frozen at −70 °C for subsequent DNA extraction. Cholesterol and triacylglycerols were measured enzymatically (Menarini Diagnostics, Firenze, Italy) with an RA-1000 Autoanalyzer (Technicon Ltd, Dublin, Ireland). HDL cholesterol was measured after precipitation of apo B–containing lipoproteins with phosphotungstic acid and magnesium (Roche Diagnostics, Madrid, Spain). LDL cholesterol was calculated according to Friedewald’s formula. Plasma apo A-I and apo B concentrations were measured by immunonephelometry (Dade Berhing, Frankfurt, Germany). The interassay CVs were as follows: cholesterol, 1.4%; triacylglycerol, 1.7%; apo A-I, 1.55%; and apo B, 4.8%.

Plasma \( \alpha \)-tocopherol, \( \gamma \)-tocopherol, lycopene, \( \alpha \)-carotene, \( \beta \)-carotene, and retinol were measured by gradient HPLC (Beckman Instruments, Palo Alto, CA) after extraction with hexane, as described elsewhere (20). Retinol acetate and tocopherol acetate were used as internal standards. The standard reference material SRM 968c from the National Institute of Standards and Technology (Gaithersburg, MD) was used as a control. The interassay CVs were as follows: \( \alpha \)-tocopherol, 5.0%; \( \gamma \)-tocopherol, 12.5%; lycopene, 5.5%; \( \alpha \)-carotene, 8.8%; \( \beta \)-carotene, 7.8%; and retinol, 4.4%.

Genomic DNA was prepared from leukocytes as described previously (13). For APOE genotyping, DNA was amplified by polymerase chain reaction by using the primers 5'CGGGCACGG-GCTGTCCAAGGAG3' and 5'CAGCGGCCTGTCCAC-GAG3'. The 244–base pair amplicon was restricted with HhaI, and the DNA fragments were separated by 8%-polyacrylamide gel electrophoresis. APOE genotype was determined by comparison with the combination of fragment sizes described by Hixson and Vernier (21).

Statistics

Statistical analyses were performed by using the software packages SPSS, version 9.0, and STATGRAPHICS PLUS, version 4.1. Analysis of variance (ANOVA) was used to examine the effects of APOE genotype after adjustment for possible confounding factors (sex, age, BMI, and city of origin). Post hoc multiple comparisons were performed with Tukey’s test. Given their skewed distribution, concentrations of triacylglycerol, \( \gamma \)-tocopherol, lycopene, \( \alpha \)-carotene, and \( \beta \)-carotene were log transformed before statistical comparison.

Pearson correlation coefficients were calculated to evaluate the correlations between fat-soluble antioxidants and lipid variables by using the log-transformed data as indicated. To ascertain the independent predictors of fat-soluble plasma antioxidants, stepwise multiple regression analysis was performed. For this, the independent variables were selected from among the anthropometric variables and plasma lipid and apolipoprotein concentrations by backward selection. To judge the relative importance of the selected independent variables in light of the noise in the data, the component effect of each predictor was calculated as coefficient × (X − Xbar), where coefficient is the estimated \( \beta \) coefficient of the variable and Xbar is the sample mean of that variable. The APOE genotype was represented as 2 dummy variables, each coded with the number (0, 1, or 2) of alleles of \( \epsilon2 \) and \( \epsilon4 \), respectively, in each genotype. An F ratio was used to test the fit of the models. Interactions were analyzed by including interaction terms in the model. A partial F was used to test the statistical significance of these terms to improve the model.

RESULTS

Plasma concentrations of lipids and apolipoproteins in the children studied are shown in Table 1. The mean cholesterol concentration in the whole group was 4.70 mmol/L. Thirty-one children (3.35%) had an LDL-cholesterol concentration > 4.14
mmol/L (160 mg/dL) and 19 children (2.05%) had HDL-cholesterol concentrations < 0.90 mmol/L (35 mg/dL), which are considered risk factors for cardiovascular disease in adults. However, none of the children had the 2 risk factors simultaneously. When lipid values in boys and girls were compared, no significant differences were found for total cholesterol or HDL-cholesterol; however, concentrations of LDL cholesterol, apo B, and total triacylglycerol were slightly but significantly higher in girls than in boys (Table 1).

Plasma concentrations of fat-soluble antioxidants are also shown in Table 1. Of the 926 children studied, 69 were occasionally given multivitamin supplements. There were no significant differences in the means of any of the measured plasma analytes, vitamins included, between children consuming and those not consuming supplements (data not shown); consequently, the results were combined. In the population as a whole, the antioxidants. The mean retinol concentration in the whole group of children after adjustment for age, sex, city of origin, and BMI. As shown in Table 2, APOE genotype influenced most of the plasma lipid concentrations as well as those of apolipoproteins. The effect on triacylglycerol was moderate, both E2 and E4 homozygotes having significantly higher concentrations than did the other groups. In contrast, total cholesterol, LDL-cholesterol, and apo B concentrations were circulating lipoprotein particles, because no significant difference in α-tocopherol concentration was noted after adjustment for apo B (21.4 ± 0.16 and 21.0 ± 0.17 μmol/L in boys and adults, respectively; P > 0.05). No sex effects were found for the rest of the antioxidants. The mean retinol concentration in the whole group was 1.02 μmol/L (range: 0.34 to 2.25 μmol/L). One child had a severely reduced retinol concentration (0.34 μmol/L), whereas 63 subjects (6.8%) had values below the clinically significant concentration of 0.7 μmol/L.

The distribution of APOE genotypes in the study population was as follows: E2/2, 0.65%; E3/2, 9.61%; E3/3, 71.27%; E4/3, 16.20%; E4/2, 0.86%; and E4/4, 1.40%. The allele frequencies were 5.9% for ε2, 85.0% for ε3, and 9.1% for ε4, which are similar to those previously reported in both Spanish (9) and other Mediterranean (7, 8) populations. Analysis of the effect of APOE genotype on plasma lipid concentrations was performed by ANOVA on the whole group of children after adjustment for age, sex, city of origin, and BMI. As shown in Table 2, APOE genotype influenced most of the plasma lipid concentrations as well as those of apolipoproteins. The effect on triacylglycerol was moderate, both E2 and E4 homozygotes having significantly higher concentrations than did the other groups. In contrast, total cholesterol, LDL-cholesterol, and apo B concentrations were

**Table 1**

Plasma concentrations of lipids, apolipoproteins, and antioxidants in children

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 926)</th>
<th>Boys (n = 441)</th>
<th>Girls (n = 485)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.795 (0.780, 0.811)</td>
<td>0.779 (0.758, 0.800)</td>
<td>0.811 (0.790, 0.832)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.70 (4.65, 4.74)</td>
<td>4.66 (4.59, 4.72)</td>
<td>4.74 (4.67, 4.80)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.80 (2.76, 2.84)</td>
<td>2.75 (2.69, 2.82)</td>
<td>2.84 (2.78, 2.90)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.51 (1.49, 1.54)</td>
<td>1.53 (1.49, 1.56)</td>
<td>1.50 (1.47, 1.54)</td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.36 (1.35, 1.37)</td>
<td>1.37 (1.36, 1.39)</td>
<td>1.35 (1.34, 1.37)</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.703 (0.694, 0.713)</td>
<td>0.689 (0.675, 0.703)</td>
<td>0.717 (0.704, 0.730)</td>
</tr>
<tr>
<td>α-Tocopherol (μmol/L)</td>
<td>21.3 (21.0, 21.5)</td>
<td>20.9 (20.5, 21.3)</td>
<td>21.6 (21.3, 22.0)</td>
</tr>
<tr>
<td>γ-Tocopherol (μmol/L)</td>
<td>2.04 (1.99, 2.09)</td>
<td>2.00 (1.93, 2.07)</td>
<td>2.08 (2.01, 2.15)</td>
</tr>
<tr>
<td>Lycopene (μmol/L)</td>
<td>0.288 (0.274, 0.303)</td>
<td>0.283 (0.264, 0.305)</td>
<td>0.292 (0.273, 0.313)</td>
</tr>
<tr>
<td>α-Carotene (μmol/L)</td>
<td>0.040 (0.047, 0.051)</td>
<td>0.050 (0.047, 0.053)</td>
<td>0.048 (0.045, 0.051)</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.201 (0.194, 0.208)</td>
<td>0.205 (0.195, 0.216)</td>
<td>0.197 (0.187, 0.206)</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>1.02 (1.00, 1.03)</td>
<td>1.00 (0.98, 1.02)</td>
<td>1.03 (1.01, 1.05)</td>
</tr>
</tbody>
</table>

1 All values are mean (95% CI) in parentheses. Apo, apolipoprotein.
2 Values are adjusted for age, city of origin, and BMI.
3 Back-transformed values from log-transformed skewed data.
4 Significantly different from boys (Student’s t test): *P < 0.05. 5P = 0.005.

**Table 2**

Plasma concentrations of lipids and apolipoproteins by apolipoprotein E (APOE) genotype in children

<table>
<thead>
<tr>
<th></th>
<th>E2/2 (n = 6)</th>
<th>E3/2 (n = 89)</th>
<th>E3/3 (n = 660)</th>
<th>E4/3 (n = 150)</th>
<th>E4/2 (n = 8)</th>
<th>E4/4 (n = 13)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>1.186(0.93, 1.50)</td>
<td>0.786(0.74, 0.83)</td>
<td>0.796(0.77, 0.81)</td>
<td>0.806(0.76, 0.84)</td>
<td>0.808(0.66, 0.98)</td>
<td>0.94E(0.80, 1.10)</td>
<td>0.006</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.024E(3.48, 4.55)</td>
<td>4.210E(4.07, 4.35)</td>
<td>4.73E(4.68, 4.78)</td>
<td>4.83E(4.72, 4.93)</td>
<td>4.19E(3.73, 4.65)</td>
<td>5.45E(5.09, 5.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.87E(1.37, 2.37)</td>
<td>2.26E(2.13, 2.39)</td>
<td>2.83E(2.78, 2.88)</td>
<td>2.99E(2.89, 3.09)</td>
<td>2.44E(2.01, 2.88)</td>
<td>3.52E(3.18, 3.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.53E(1.26, 1.81)</td>
<td>1.58E(1.50, 1.65)</td>
<td>1.52E(1.50, 1.55)</td>
<td>1.45E(1.39, 1.50)</td>
<td>1.37E(1.13, 1.61)</td>
<td>1.49E(1.30, 1.68)</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.46E(1.31, 1.61)</td>
<td>1.41E(1.37, 1.44)</td>
<td>1.36E(1.35, 1.38)</td>
<td>1.33E(1.30, 1.36)</td>
<td>1.27E(1.14, 1.40)</td>
<td>1.43E(1.33, 1.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.38E(0.27, 0.49)</td>
<td>0.56E(0.54, 0.59)</td>
<td>0.71E(0.70, 0.72)</td>
<td>0.76E(0.74, 0.78)</td>
<td>0.60E(0.51, 0.70)</td>
<td>0.90E(0.83, 0.98)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

1 All values are mean; 95% CI in parentheses. Plasma lipid and apolipoprotein concentrations were adjusted for age, sex, city of origin, and BMI. Apo, apolipoprotein. Means within a row with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc test).
2 One-way ANOVA F test.
3 Back-transformed values from log-transformed skewed data.
TABLE 3
Plasma concentrations of fat-soluble vitamins by apolipoprotein E (APOE) genotype in children.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Calcium (µmol/L)</th>
<th>α-Tocopherol</th>
<th>γ-Tocopherol</th>
<th>Lycopene</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/2</td>
<td>6</td>
<td>0.021±0.002</td>
<td>0.052±0.002</td>
<td>0.050±0.002</td>
<td>0.002±0.273</td>
<td>0.010±0.016</td>
<td>0.097±0.255</td>
<td>0.97±0.97</td>
</tr>
<tr>
<td>E3/2</td>
<td>89</td>
<td>0.024±0.002</td>
<td>0.024±0.002</td>
<td>0.050±0.002</td>
<td>0.002±0.273</td>
<td>0.010±0.016</td>
<td>0.097±0.255</td>
<td>1.01±1.01</td>
</tr>
<tr>
<td>E3/3</td>
<td>660</td>
<td>0.024±0.002</td>
<td>0.024±0.002</td>
<td>0.050±0.002</td>
<td>0.002±0.273</td>
<td>0.010±0.016</td>
<td>0.097±0.255</td>
<td>1.02±1.02</td>
</tr>
<tr>
<td>E4/4</td>
<td>13</td>
<td>0.024±0.002</td>
<td>0.024±0.002</td>
<td>0.050±0.002</td>
<td>0.002±0.273</td>
<td>0.010±0.016</td>
<td>0.097±0.255</td>
<td>0.98±0.98</td>
</tr>
</tbody>
</table>

Plasma antioxidant concentrations were adjusted for age, sex, city of origin, and BMI. Minimum (min) and maximum (max) correspond to original, not transformed, values. 95% CI 0.79–1.15 0.96–1.06 1.00–1.03 0.98–1.05 0.032–1.120 0.049–0.297 0.83–1.13 0.93–1.17

1 Plasma antioxidant concentrations were adjusted for age, sex, city of origin, and BMI. Minimum (min) and maximum (max) correspond to the original data. Means within a row with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc test).
2 One-way ANOVA F test.
3 Back-transformed values from log-transformed skewed data.

TABLE 4
Pearson correlation coefficients between plasma concentrations of fat-soluble antioxidants and lipids and apolipoproteins in children.

<table>
<thead>
<tr>
<th>Triacylglycerol</th>
<th>Cholesterol</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Apo B</th>
<th>Apo A-I</th>
<th>LDL-C:Apo B</th>
<th>HDL-C:Apo A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>0.1444</td>
<td>0.4646</td>
<td>0.3584</td>
<td>0.1981</td>
<td>0.4896</td>
<td>-0.2060</td>
<td>-0.1393</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>NS</td>
<td>0.1109</td>
<td>0.0965</td>
<td>NS</td>
<td>0.1344</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lycopene</td>
<td>NS</td>
<td>0.1417</td>
<td>0.1034</td>
<td>0.0896</td>
<td>0.1196</td>
<td>0.1656</td>
<td>NS</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>NS</td>
<td>0.1836</td>
<td>0.1569</td>
<td>0.0895</td>
<td>0.1683</td>
<td>0.1023</td>
<td>NS</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>NS</td>
<td>0.2509</td>
<td>0.2157</td>
<td>0.1014</td>
<td>0.2303</td>
<td>0.1287</td>
<td>NS</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.0698</td>
<td>0.1414</td>
<td>0.0829</td>
<td>0.0963</td>
<td>0.1627</td>
<td>0.1691</td>
<td>-0.1023</td>
</tr>
</tbody>
</table>

1 n = 926. Apo, apolipoprotein.
2 Log-transformed skewed data.
3 P < 0.001.
4 P < 0.01.
5 P < 0.05.
variables: total triacylglycerol, which provides an estimate of energy consumption (data not shown). A positive correlation between fat-soluble antioxidants and fat, protein, fiber, and saturated, monounsaturated, or polyunsaturated fatty acids (data not shown). Moreover, the correlation coefficients were higher with apolipoproteins than with their cholesterol counterpart, ie, apo B versus LDL cholesterol and apo A-I versus HDL cholesterol, for each fat-soluble vitamin studied, including retinol. Plasma concentrations of retinol, a vitamin transported bound to retinol-binding protein rather than in lipoproteins in fasting plasma, showed a positive correlation with all lipids and apolipoproteins studied and a negative correlation with the ratio of LDL cholesterol to apo B (Table 4).

Food consumption in children was estimated on the basis of a food-frequency questionnaire completed by their parents; these results are published elsewhere (22, 23). No significant correlations were found between fat-soluble plasma antioxidants and any of the major diet components considered, namely, energy, protein, fat, carbohydrates, fiber, and saturated, monounsaturated, or polyunsaturated fatty acids (data not shown). Moreover, neither tocopherol nor retinol plasma concentrations were significantly correlated with estimated vitamin E or vitamin A dietary consumption (data not shown).

We next analyzed the predictors of plasma fat-soluble antioxidant concentrations by multiple linear regression and backward selection. The following variables were included as independent variables: total triacylglycerol, which provides an estimate of VLDL cholesterol (24); LDL cholesterol; HDL cholesterol; apo A-I; and apo B plasma concentrations. The selected variables and their standardized coefficients (β) and P values, along with the \( R^2 \) values of the models are given in Table 5. For \( \alpha \)-tocopherol, the identified predictors were total triacylglycerol, apo A-I, and apo B, with the model explaining as much as 27.6% of the variability in plasma \( \alpha \)-tocopherol concentrations. In the case of \( \gamma \)-tocopherol, the only identified predictor was apo B. Lycopene, \( \alpha \)-carotene, and \( \beta \)-carotene concentrations were predicted by both apo A-I and apo B, although the magnitude of the effects of these apolipoproteins was much lower than in the case of \( \alpha \)-tocopherol, as indicated by the \( \beta \) coefficients. Retinol concentration was predicted by apo A-I and apo B, and, to a lesser extent, by LDL cholesterol. Finally, the resulting models for \( \gamma \)-tocopherol, lycopene, carotenes, and retinol explained a much lower variability of their respective concentrations than in the case of \( \alpha \)-tocopherol.

The results of the multivariate analysis prompted us to adjust fat-soluble antioxidant concentrations for triacylglycerols, apo A-I, and apo B to evaluate a possible lipoprotein-independent effect of \( APOE \) genotype. As shown in Table 6, a significant effect of \( APOE \) genotype was still noted for vitamin E. In the case of \( \alpha \)-tocopherol, the highest concentration was found in \( E2/2 \) individuals, whereas no significant differences were observed between the other genotypes. A similar trend was observed for

### Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Selected Predictors</th>
<th>( \beta )</th>
<th>( P )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>Triacylglycerol</td>
<td>1.1161</td>
<td>0.009</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Apo A-I</td>
<td>3.8405</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo B</td>
<td>12.4992</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>Apo B</td>
<td>0.3514</td>
<td>&lt;0.001</td>
<td>1.8</td>
</tr>
<tr>
<td>Lycopene</td>
<td>Apo A-I</td>
<td>0.6309</td>
<td>&lt;0.001</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Apo B</td>
<td>0.5799</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>( \alpha )-Carotene</td>
<td>Apo A-I</td>
<td>0.3448</td>
<td>0.003</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Apo B</td>
<td>0.7595</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td>Apo A-I</td>
<td>0.3346</td>
<td>&lt;0.001</td>
<td>6.7</td>
</tr>
<tr>
<td>Retinol</td>
<td>LDL cholesterol</td>
<td>-0.0493</td>
<td>0.005</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Apo A-I</td>
<td>0.1849</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apo B</td>
<td>0.4013</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

1 The model included the following independent variables: triacylglycerol, LDL cholesterol, HDL cholesterol, apolipoprotein (apo) A-I, apo B, city of origin, BMI, and age. In the backward selection, variables were removed for \( F \) values < 5. Only statistically significant predictors are shown.
2 Log-transformed skewed data.

### Table 6

<table>
<thead>
<tr>
<th>( E2/2 ) (n = 6)</th>
<th>( E3/2 ) (n = 89)</th>
<th>( E3/3 ) (n = 660)</th>
<th>( E4/3 ) (n = 150)</th>
<th>( E4/2 ) (n = 8)</th>
<th>( E4/4 ) (n = 13)</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>26.5(^a) (23.8, 29.2)</td>
<td>20.8(^b) (20.1, 21.5)</td>
<td>21.3(^b) (21.1, 21.6)</td>
<td>21.4(^b) (20.9, 21.9)</td>
<td>21.7(^b) (19.4, 23.9)</td>
<td>19.0(^b) (17.2, 20.8)</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>3.10(^b) (2.2, 4.2)</td>
<td>1.90(^b) (1.75, 2.07)</td>
<td>2.06(^b) (2.0, 2.12)</td>
<td>2.05(^b) (1.92, 2.18)</td>
<td>1.81(^b) (1.39, 2.36)</td>
<td>1.84(^b) (1.49, 2.27)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.383 (0.206, 0.710)</td>
<td>0.237 (0.101, 0.380)</td>
<td>0.299 (0.282, 0.317)</td>
<td>0.278 (0.246, 0.314)</td>
<td>0.253 (0.150, 0.429)</td>
<td>0.237 (0.156, 0.360)</td>
</tr>
<tr>
<td>( \alpha )-Carotene</td>
<td>0.061 (0.035, 0.106)</td>
<td>0.043 (0.037, 0.050)</td>
<td>0.050 (0.048, 0.053)</td>
<td>0.049 (0.043, 0.054)</td>
<td>0.034 (0.021, 0.057)</td>
<td>0.051 (0.035, 0.075)</td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td>0.181 (0.118, 0.276)</td>
<td>0.204 (0.182, 0.229)</td>
<td>0.204 (0.196, 0.212)</td>
<td>0.193 (0.177, 0.210)</td>
<td>0.155 (0.108, 0.222)</td>
<td>0.189 (0.142, 0.252)</td>
</tr>
<tr>
<td>Retinol</td>
<td>1.05 (1.05, 1.22)</td>
<td>1.03 (0.99, 1.08)</td>
<td>1.02 (1.00, 1.03)</td>
<td>1.01 (0.97, 1.04)</td>
<td>1.02 (0.87, 1.17)</td>
<td>0.99 (0.87, 1.11)</td>
</tr>
</tbody>
</table>

1 All values are \( \bar{x} \); 95% CI in parentheses. Plasma antioxidant concentrations were adjusted for triacylglycerol, apolipoprotein (apo) A-I, apo B, city, age, sex, BMI. Means within a row with different superscript letters are significantly different, \( P < 0.05 \) (Tukey’s post hoc test).
2 One-way ANOVA \( F \) test.
3 Back-transformed values from log-transformed skewed data.
gamma-tocopherol. Significant associations between lycopene or carotenoids and APOE genotype were no longer observed after adjustment for apo A-I and apo B. This indicates that the differences in carotenoid concentrations between individuals with different APOE genotypes are mainly attributable to the number of circulating lipoprotein particles. Similarly, no significant variation in retinol concentrations was found between different APOE genotypes after adjustment for significant covariates.

We examined a possible interaction between APOE genotype and lipoprotein-related variables in determining the concentration of alpha-tocopherol, the antioxidant most influenced by such variables. To this end, the effects of the e2 and e4 alleles, as compared with the e3 allele, as well as the interacting terms of the e2 and e4 alleles with triacylglycerol, apo A-I, or apo B, were determined. Significant interactions of APOE genotype with triacylglycerol (P < 0.05) and apo B (P < 0.05), but not with apo A-I, were found. Regarding the B coefficients for the individual interacting terms, a significant interaction was found only between the e2 allele and triacylglycerol (β = 2.318, P < 0.05). To further examine the effect of triacylglycerol and apo B on alpha-tocopherol concentrations in each individual genotype, a multiple regression analysis was performed, but now stratifying for APOE genotype. Because of the small number of subjects in certain genotypes, they were grouped into E2 carriers (comprising E2/2 and E3/2 subjects), E3 homozygotes, and E4 carriers (E4/4 and E4/3 subjects); E4/2 subjects were excluded from this analysis. The component effects of triacylglycerols and apo B are shown in Figure 1. Apo B was identified as a significant predictor of alpha-tocopherol in all groups, However, clear differences were found for the other lipoprotein-related variables. Triacylglycerol was identified as a significant predictor of alpha-tocopherol concentrations in the E2 subjects but not in the E3 and E4 groups (Figure 1), which confirms the interaction between the e2 allele and triacylglycerol. In fact, the association between triacylglycerol and alpha-tocopherol in the whole population (Table 5) was attributable specifically to the E2 subjects, because it did not persist after this group was eliminated from the multivariate analysis (data not shown).

**DISCUSSION**

Few published reports exist on fat-soluble antioxidant plasma concentrations in prepubertal children in developed countries; the work presented here is the first study of such characteristics conducted in Spain. Total vitamin E concentrations were similar to values reported in Italian children (25) but were higher than values found in other countries (26–33), with only 4 children (0.4%) having vitamin E concentrations sufficiently low to be considered deficient. Other authors reported a prevalence of vitamin E deficiency in children; ranging from none in France (28) to >30% in the United States (34, 35). The variability in plasma lipid concentrations among populations may contribute to these differences. Furthermore, the alpha-tocopherol/cholesterol + triacylglycerol ratio in our population of children remained relatively high (3.87 ± 0.67 μmol/mmol; ± SD), and none of the children studied had a ratio <1.59, which is considered an accurate index of the risk of vitamin E deficiency (36).
The plasma retinol concentration in Spanish children appears to be similar to that in children in other countries when the same age groups are compared (27, 28, 30, 33, 37–39). In our population, only one child (0.1%) had a retinol concentration <0.35 μmol/L, which could be considered to indicate a high risk of anemia (40), and 6.8% had values below the clinically significant concentration of 0.7 μmol/L. In other countries, the proportion of children with concentrations <0.7 μmol/L was reported to be between 0% and 11.6% (27, 28, 30, 33, 37, 38). In general, children from less developed areas had, on average, lower plasma retinol concentrations than did children with higher living standards. Moreover, we found a positive correlation between the plasma retinol concentration and BMI (r = 0.1531, P < 0.001) in our study population. Thus, both living standards and lower BMI may contribute to explaining the relatively high proportion of subjects in our population with retinol concentrations <0.7 μmol/L (6.8%).

In general, carbohydrate intake in this sample of children was lower, whereas protein and fat intakes were higher, than current dietary recommendations (22). Dietary consumption of vitamin A (667 ± 196 μg/d; x ± SD) and vitamin E (11.0 ± 4.1 mg/d; x ± SD) was well above current recommendations. However, we did not find any significant correlation between fat-soluble plasma antioxidants and the major nutrients or vitamins consumed. Other authors also reported no association between dietary intake and plasma concentrations of retinol in well-nourished populations (41, 42). The results of studies of adult populations that addressed the influence of diet on serum tocopherol are conflicting: some authors reported a small association of dietary intake and plasma concentrations of retinol in well-nourished populations. Other authors also reported no association between dietary intake and plasma concentrations of retinol in well-nourished populations (41, 42). Other authors also reported no association between dietary intake and plasma concentrations of retinol in well-nourished populations (41, 42). The reasons for this large variation in plasma antioxidants were strongly correlated with those of plasma lipids and especially with the most abundant apolipoproteins, apo A-I and apo B. In multivariate linear regression analysis, apo A-I and apo B were identified as predictors instead of the cholesterol moiety of the respective lipoproteins (ie, HDL and LDL) for all the antioxidants measured. This indicates that the principal determining factor of the plasma concentration of these antioxidants is the number of lipoprotein particles carrying them, rather than the lipid (cholesterol) content of the lipoproteins.

When the role of the apo E polymorphism in determining the variability in antioxidant concentrations was assessed, the results essentially agreed with other reports in children (51–54). Concentrations of γ-tocopherol, lycopene, α-carotene, and, most significantly, α-tocopherol varied as a function of APOE genotype (Table 3). However, these differences were attenuated or disappeared after adjustment for lipoprotein-related variables (Table 6). Despite this, interactions of the polymorphism with triacylglycerol and apo B in determining the concentration of α-tocopherol were found. Taking into account the respective β coefficients and plasma concentrations of the predictors, the main predictor of the α-tocopherol concentration in every APOE genotype was apo B, which mainly reflects the number of circulating LDL particles. In the E2 carriers, but not in the other subjects, triacylglycerol was also identified as a predictor of the α-tocopherol concentration. Given that in the E2 subjects LDL cholesterol and apo B concentrations are lower than in the other groups, such an association with triacylglycerols suggests a redistribution of α-tocopherol, shifting from LDL to VLDL. This phenomenon was more marked in the E2/2 subjects, whose α-tocopherol and triacylglycerol concentrations were the highest and whose LDL cholesterol and apo B were the lowest. This is probably the result of the tocopherol transfer protein–mediated incorporation of α-tocopherol into VLDL (55, 56), the production of which is increased in E2 homozygotes (57).

Both apo A-I and apo B were identified as predictors of carotenoid concentrations, although the effect of these apolipoproteins on the concentration of carotenoids was much lower than on the concentration of α-tocopherol. Moreover, this contribution was essentially the same in E3/3 subjects as in E2 or E4 carriers (data not shown). Goulinet and Chapman (58) showed that whereas carotenoids are present in all lipoprotein classes, the content in VLDL is much lower than in other classes, which is consistent with plasma triacylglycerol not being identified as a predictor of carotenoid concentrations.

Apo E isoforms have been shown to confer differential protection against free radicals, the highest activity being exhibited by apo E2 and the lowest by the E4 isoform (15, 16). After adjustment for lipids, the mean plasma α-tocopherol concentration in E4 carriers was not significantly different from that in E3 homozygotes, both in prepubertal children (present work) and in adults (19). However, the threshold of vitamin E deficiency and the appropriateness of vitamin E supplementation in E4/4 individuals, who are at high risk of various degenerative diseases (5, 59), should be reevaluated in clinical assays because of the decreased antioxidant capacity of apo E4.

In summary, the association of the APOE genotype with lipophilic antioxidant concentrations in the population of children studied here was mainly dependent on the effect of the polymorphism on lipoprotein concentrations. Apo B is the main predictor...
of α-tocopherol concentrations; however, an interaction exists between certain lipoprotein-related variables and the APOE genotype. In the E2 carriers, but not in the other subjects, triacylglycerol levels determine the variability of α-tocopherol concentrations. This suggests that the content of this antioxidant in each lipoprotein class may vary according to the specific APOE genotype, a possibility that should be confirmed by future studies.

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HO performed the vitamin determinations and sample collection. PC contributed to the vitamin determinations. DG-C contributed to the writing of the manuscript. CB and MB contributed to sample collection and performed APOE genotyping. FR-A contributed to diet analysis. MdO contributed to the design and general management of the Four Provinces Study. MAL contributed to biochemical analysis, supervised the study, and wrote the manuscript. None of the authors had any financial or personal conflicts of interest.

REFERENCES


