MS #20298 Version 2

TITLE: The apolipoprotein E gene promoter (-219G/T) polymorphism increases LDL cholesterol levels and susceptibility to oxidation in response to a saturated fat-rich diet.

AUTHORS: Juan Antonio Moreno, Francisco Pérez-Jiménez, Carmen Marín, Purificación Gómez, Pablo Pérez-Martínez, Rafael Moreno, Cecilia Bellido, Francisco Fuentes, José López-Miranda.

Lipids and Atherosclerosis Research Unit. Reina Sofía University Hospital. Córdoba. Spain.

Correspondence and reprint request to: José López-Miranda. Unidad de Lípidos y Arteriosclerosis. Hospital Universitario Reina Sofía. Avda Menéndez Pidal, s/n. 14004 Córdoba, Spain. Phone: 34-957-218250 FAX: 34-957-218250

E-mail: md1lomij@uco.es

This work was supported by research grants from the CICYT (SAF 01/2466-C05 04, SAF 01/0366), the Spanish Ministry of Health (FIS, 98/153, FIS 01/0449 and FIS 99/0949), Fundación Cultural "Hospital Reina Sofía-Cajasur", Consejería de Salud, Servicio Andaluz de Salud (99/116, 00/212, 01/243, 99/165, 00/39 and 01/239), and Consejería de Educación, Plan Andaluz de Investigación, Universidad de Córdoba.

Short running head: Interaction of diet and -219G/T apoE polymorphism.

ABSTRACT

Background: The *apolipoprotein E* (apoE) gene promoter (-219G/T) polymorphism has been associated with increased risk of myocardial infarction, premature coronary heart disease and decreased plasma ApoE concentrations.

Objective: To determine whether this polymorphism modifies LDL oxidation susceptibility and lipid response to the content and quality of dietary fat in healthy subjects.

Design: 55 healthy apoE 3/3 men (7 GG, 38 GT and 10 TT) were subjected to three dietary periods, each lasting four weeks. The first was a saturated fatty acid (SFA)-rich diet (38% fat, 20% SFA, 12% monounsaturated fatty acid (MUFA), 47% carbohydrates (CHO)), followed by a CHO-rich diet (30% fat, <10% SFA, 12% MUFA, 55% CHO) or a MUFA-rich diet (38% fat, <10% SFA, 22% MUFA, 47% CHO) in a randomized crossover design. At the end of each diet period, LDL oxidation susceptibility, lipids and lipoproteins were measured.

Results: Compared with carriers of the G allele, TT subjects have significantly ($p<0.05$) lower lag time (LT) after SFA diet. The replacement of SFA diet by CHO or MUFA diets induces a higher increase ($p<0.05$) of LT in TT subjects when compared to GG and GG subjects. Carriers of the T allele have higher LDL-C ($p<0.05$) and ApoB ($p<0.05$) plasma concentration after SFA diet when compared to GG subjects. Compared with GG subjects, carriers of the T allele have a significantly ($p<0.05$) higher decrease in LDL-C and ApoB when they changed from SFA to CHO diet.

Conclusion: The -219G/T polymorphism could partially explain differences in individual response to diet.

Key words: *apolipoprotein E* gene promoter (-219G/T) polymorphism, dietary intervention, LDL oxidation, LDL cholesterol, cardiovascular risk.

INTRODUCTION

Apolipoprotein E (ApoE) is a structural component of several lipoproteins and serves as a ligand for the low-density lipoprotein (LDL)-receptor and the LDL receptor-related protein (1,2). Therefore, ApoE plays an important role in lipid metabolism by both promoting efficient uptake of triglyceride-rich lipoproteins from the circulation and by taking part in the cellular cholesterol efflux and reverse cholesterol transport (3). However, such functions are not uniformly effective because ApoE is present in the population in three main isoforms (ApoE2, ApoE3 and ApoE4), which determine ApoE concentrations and differ in their affinity to bind to the specific receptors (4,5). In addition to the effects on plasma lipids, the ApoE has been reported to influence LDL oxidation *in vivo* and *in vitro*. ApoE has antioxidant activity and this activity differs in extent according to isoform and may be related to the ability of ApoE to sequester copper *in vitro* (6). Other studies have shown that ApoE knockout mice are highly susceptible to developing atherosclerosis (7), at least partly because their LDL is more susceptible to oxidation (8), and also lose their normal resistance to cholesterol feeding. This effect is reversible by antioxidant supplementation of their diet (9).

In recent years, the interaction between lipoprotein responsiveness to dietary intervention and apoE genotypes has been analyzed, however the results have been controversial (10). Whereas some studies have found a pronounced dietary responsiveness among apoE4 carriers, others have reported no difference in response across apoE genotypes to changes in dietary fat or cholesterol content. (11-14). This fact suggests that other genetic or environmental factors, are likely to be responsible for the association of the apoE gene and individual response to diet.

In accordance with this hypothesis, polymorphisms in the proximal promoter region of the *apoE* gene have recently been described at positions -491 A/T, -427 T/C and -219 G/T

(15,16). In particular, there are experimental evidences, in vitro and in vivo, indicating that the *apoE* gene promoter (-219G/T) polymorphism produces variations in the transcriptional activity of the gene. Specifically, the -219G allele showed a higher transcriptional activity than the -219T allele (15). A study of a European population including control individuals and multiinfarct patients has shown that the *apoE* gene promoter (-219G/T) polymorphism is also associated with differential plasma ApoE concentration (17), illustrating the fact that this polymorphism influences *apoE* expression in vivo. Furthermore, the -219T allele is associated with an increased risk of myocardial infarction (MI) (17) and premature coronary heart disease (CHD) (18).

The mechanisms via which the -219T allele of the *apoE* gene enhances atherothrombosis remain to be elucidated. In a previous study, we observed that the presence of this polymorphism determines serum ApoE levels and influences the metabolism of triacylglycerol-rich lipoproteins during the postprandial period (19). In other study, the -219T allele was associated with insulin resistance (18). However, in the study by Lambert et al (17) the -219G/T polymorphism was not associated with plasma lipid or lipoprotein concentrations.

Thus, the aim of this study was to determine whether the *apoE* gene promoter (-219G/T) polymorphism modifies LDL oxidation susceptibility and lipid response to the quantity and quality of dietary fat in healthy apoE3/E3 men.

METHODS

A group of 55 men (7 with the -219GG genotype, 38 with the -219GT genotype and 10 with the -219TT genotype), were recruited from among students at the University of Cordoba. As APOE genotypes have been implicated in a variable lipid response to dietary changes, consequently we have studied the effect of the *apoE* gene promoter (-219G/T) polymorphism in apoE3/E3 subjects, to the exclusion of other apoE isoforms. The subjects had a mean age (± SD) of 21± 0.8 years. Informed consent was obtained from all participants. All subjects underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrollment. Subjects showed no evidence of any chronic disease (hepatic, renal, thyroid, or cardiac dysfunction), obesity or unusually high levels of physical activity (e.g. sports training). None of the subjects had a family history of premature coronary artery disease or had taken medications or vitamin supplements in the 6 months prior to the study. Physical activity and diet, including alcohol consumption, were recorded in a personal diary for 1 week and the data were used to calculate individual energy requirements. Mean body mass index (BMI) was 22.86 ± 0.28 Kg/m^2 (mean ± SD) at the onset of the study and remained constant throughout the experimental period. Subjects were encouraged to maintain their regular physical activity and lifestyle and were asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits and alcohol consumption or intake of foods not included in the experiment design. The study protocol was approved by the Human Investigation Review Committee at the Reina Sofia University Hospital.

Diets

The study design included an initial 28-day period during which all subjects

consumed a saturated fatty acid (SFA)-rich diet, with 15% protein, 47% carbohydrate and 38% fat (20% SFA, 12% monounsaturated fatty acid (MUFA) and 6% polyunsaturated fatty acid (PUFA)). After this period, volunteers were randomly assigned to 1 of 2 diet sequences. 28 subjects received a MUFA-rich diet containing 15% protein, 47% carbohydrates and 38% fat (<10% SFA, 6% PUFA, 22% MUFA) for 28 days. This diet was followed for 28 days by consumption of a carbohydrate (CHO)-rich diet containing 15% protein, 55% carbohydrates and <30% fat (<10% SFA, 6% PUFA, 12% MUFA). The other 27 subjects consumed the CHO diet before the MUFA diet. The cholesterol content remained constant (under 300 mg/day) during the three periods. 80% of the MUFA diet was provided by virgin olive oil, which was used for cooking, salad dressing and as a spread. Carbohydrate intake of the CHO diet was based on the consumption of biscuits, jam and bread. Butter and palm oil were used during the SFA dietary period.

The composition of the experimental diets was calculated using the United States Department of Agriculture (20) food tables, and the Spanish food composition tables for local foodstuffs (21). All meals were prepared in the hospital kitchen and were supervised by a dietitian. Lunch and dinner were consumed in the hospital dining room, whereas breakfast and an afternoon snack were eaten in the medical school cafeteria. Fourteen menus were prepared with regular solid foods and rotated during the experimental period. Duplicate samples from each menu were collected, homogenized, and stored at –70ºC. Protein, fat, and carbohydrate contents of the diet were analyzed by standard methods (22). Dietary compliance was verified by analyzing the fatty acids in LDL cholesterol esters at the end of each dietary period (23). The study took place from January through March to minimize seasonal effects and academic stress.

Analysis of fatty acids in LDL cholesterol esters.

Analysis of fatty acids was examined by gas liquid chromatography (GLC). Lipids were transmethylated as previously described (23). The resulting fatty acid methyl esters were eluted with hexane and analyzed by GLC using a model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and an Omegawax 320 fused-silica capillary column (30m x 0.32 mm I.D., 0.25 μm film), obtained from Supelco (Bellafonte, PA, USA) with helium as the carrier gas.

Lipid analysis and biochemical determinations

Venous blood samples were collected into EDTA-containing (1g/L) tubes from all subjects after a 12-h overnight fast at the beginning of the study and at the end of each dietary period. Plasma was obtained by low speed centrifugation for 15 min at 4°C within 1h of venipuncture. To reduce interassay variation, plasma was stored at -80°C and analyzed at the end of the study. Plasma cholesterol and triacylglycerol levels were determined by enzymatic techniques (24,25). HDL cholesterol was determined after precipitation with fosfowolframic acid (26). Apo A-I and B were determined by immunoturbidimetry (27). LDL cholesterol concentration was calculated using the Friedewald formula (28).

Oxidation of LDL

LDL were isolated from fresh plasma samples by sequential ultracentrifugation using a Beckman model LE-70 ultracentrifuge with a Type NVT65 rotor (Beckman Palo Alto, CA, USA) for 2 h at 65,000 rpm at a temperature of 4 ºC. The formation of conjugated dienes was measured by incubating 100 μg LDL protein with 5 μmol/L $CuSO_4$ in 1.0 mL PBS medium. The absorbance at 234 nm was measured continuously every 5 minutes for 4 hours at 37ºC in a spectophotometer as previously described (29). Results are expressed as the duration of the

lag time before propagation of the LDL oxidation reaction determined by the absolute increase in absorbance above the initial value.

DNA amplification and genotyping

Genomic DNA extraction and a*poE* E2/E3/E4 (30) and –219G/T (12-14) genotypes were determined as previously described. Digested DNA was separated by electrophoresis on an 8% non-denaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining. Samples containing the T allele of –219G/T polymorphism were amplified a second time to verify the genotype.

Statistical analysis

We used the analysis of variance (ANOVA) for repeated measures to test for effects of the *apoE* gene promoter (-219G/T) polymorphism on plasma total cholesterol, LDL cholesterol, HDL cholesterol, trialcylglycerol, apo A-I, and apo B concentration in each dietary stage. When statistical significance was found, Tukey's post-hoc comparison test was used to identify between-group differences. Statistical analyses were carried out using the SPSS statistical software, version 8.0 (SPSS Inc, Chicago).

RESULTS

Significant differences were not observed in any of the variables studied when we compared the baseline characteristics of subjects who were homozygous for the G allele (GG, n=7), subjects heterozygous for the T allele (GT, n=38) and subjects homozygous for the T allele (TT, n=10) in the *apoE* gene promoter (-219G/T) polymorphism (**Table 1**). The composition of the mean daily intake of the participants is shown in **Table 2**. Analysis of LDL-cholesterol esters obtained after each dietary period showed good adherence in the different intervention stages. After the SFA-diet period, we observed a significantly greater ($p<0.005$) increase in palmitic acid in the LDL-cholesterol esters than were observed after the CHO and MUFA diets: 27.3 ± 1.4 % compared with 19.8 ± 3.9 % and 15.2 ± 0.4 %, respectively. A significantly greater ($p<0.05$) increase in oleic acid in the cholesterol esters was also seen after the MUFA diet (50.3 ± 4.7 %) than after the CHO diet (38.8 ± 9.0 %), but not after the SFA diet (47.2 ± 4.4 %).

The lag time before propagation of the LDL oxidation reaction and concentrations of total cholesterol, LDL-C, HDL-C, apo A-1 and apo B after the three diets are shown in **Table 3**. Changes in diet were associated with significant ($p<0.05$) higher lag times and significant lower concentration of total ($p<0.001$), LDL ($p<0.001$), and HDL cholesterol ($p<0.05$); apoA-I ($p<0.05$), apoB ($p<0.001$) after the CHO and MUFA diets. However, in comparison with the MUFA diet, the CHO diet was associated with significant lower lag times ($p<0.01$) and significant lower plasma concentrations of HDL-C ($p<0.05$) and apoA-I ($p<0.01$). Significant differences were not observed in triacylglycerol concentrations after the different diets ($p=0.695$).

A significant effect on apoB ($p=0.038$), HDL cholesterol ($p=0.005$) and LDL cholesterol ($p=0.048$) concentration was observed for the interaction between this

polymorphism and diet (**Table 3**). Carriers of the T allele were observed to have significantly ($p<0.05$) higher levels of ApoB and LDL cholesterol after the SFA diet compared to subjects homozygous for the G allele. Thus, in TT and GT subjects, the decrease in ApoB plasma concentrations was significantly ($p=0.049$) greater when they changed from a SFA diet to a CHO one (-14% TT and -16% GT) compared to GG subjects (-5%). In carriers of the T allele, the decrease in LDL cholesterol concentrations was significantly ($p=0.048$) higher when they changed from a SFA to a CHO diet (-21% TT and -17% GT) compared to subjects homozygous for the G allele (-5%). However, when the SFA diet was compared with the MUFA diet, no significant differences in ApoB or LDL cholesterol levels were observed between genotypes.

The *apoE* gene promoter (-219G/T) polymorphism has a significant effect on the lag time before propagation of the LDL oxidation reaction ($p=0.025$). Compared with carriers of the G allele, TT subjects have significantly ($p<0.05$) lower lag times after the SFA diet. A significant effect on lag times ($p=0.032$) was observed for the interaction between this polymorphism and diet. Thus, the replacement of a SFA diet by a CHO diet induces a higher increase ($p=0.047$) of the lag times in TT subjects (14.2 min, 72%) as compared to GG subjects (9.50 min, 14%). In the same way, a significantly ($p<0.001$) higher increase in lag times (29.25 min, 154%) was observed in TT subjects as compared to carriers of the G allele (16.25 min, 30% GG and 9.33 min, 34% GT) when the SFA diet was compared to the MUFA diet. In addition we have observed a significant correlation ($r=-0.27$, $p=0.005$) between the increase in LDL cholesterol and the decrease in lag time.

DISCUSSION

Our results show that the presence of the T allele in the apoE gene promoter (-219G/T) polymorphism increases the susceptibility of plasma LDL to oxidative modifications and enhances the response of ApoB and LDL cholesterol to the presence of SFA in the diet of healthy men. Previous studies suggest that differences in individual response to diet exist. Therefore, the influence of the genetic loci of the principal apolipoproteins such as the AI-CIII-AIV complex and the apoE gene have been studied. The allelic variables of these genes influence the degree of response by both HDL and LDL cholesterol (31,32). Thus the hyperresponse of LDL cholesterol concentrations associated with the E4 allele occurred only when the fat content in diet varied (33). However, the inconsistencies observed in several studies (10-14) suggest that other genetic or environmental factors may interact with the apoE gene in determining the individual response to diet. Thus, in our study we have observed that carriers of the T allele, in the apoE gene promoter (-219G/T) polymorphism, showed a higher response of LDL cholesterol and ApoB when consumed a SFA-rich diet. Thus, in our study we have observed that carriers of the T allele, in the apoE gene promoter (-219G/T) polymorphism, showed a higher response of LDL cholesterol and ApoB when consumed a SFA-rich diet. Furthermore, in carriers of the T allele, the decrease in LDL-C and ApoB was significantly ($p<0.05$) higher when they changed from a SFA to a CHO diet.

The -219T allele is associated with an increased risk of MI, premature CHD and decreased plasma ApoE concentrations (17,18). These facts may suggest that the basal ability of cells to synthesize and secrete ApoE may be of particular relevance in atherothrombosis. Recent studies have shown that the genetic expression of the ApoE modifies the lipoprotein metabolism in animals. In transgenic mice, the increase of ApoE expression produced an increase of up to 300% in the uptake of chylomicron remnant, VLDL and LDL (34). In

addition, uptake of apoB containing lipoproteins was also increased by the LDL receptor, probably through an interaction with the LDL receptor-related protein. On the contrary, the total suppression of its expression results in a massive accumulation of cholesterol-rich VLDL-like remnants and also LDL-like particles. The higher plasma LDL concentrations and the prolonged circulation of these atherogenic lipoproteins in the plasma of the apoE knockout mice (E°) cause an oxidative stress which is associated with the increased susceptibility of the lipoprotein to oxidation, specially when the animals are stressed by an atherogenic diet. This phenomenon could explain our results, where TT subjects, with lower ApoE plasma concentration (17,19) and higher LDL plasma concentration, have a higher susceptibility of plasma LDL to oxidative modifications when consumed a SFA-rich diet. In accordance with this hypothesis, we have observed a significant correlation between the increase in LDL cholesterol and decrease in lag time. In addition, ApoE is able to protect against atherosclerosis because it acts as an antioxidant (35). Thus, studies in E° mice have reported an increase in LDL oxidation (8,36). Moreover, E° mice that were fed with an antioxidant-rich diet showed greater resistance to in vitro oxidation and the extent of atherosclerosis was significantly reduced compared to nontreated mice. E° mice fed with high-fat, low-cholesterol diets enriched with olive oil are associated with a reduction in atherosclerotic lesions and a decrease in hepatic lipid peroxidation (37).

We have recently observed that the presence of the (-219G/T) polymorphism influenced the metabolism of triacylglycerol-rich lipoproteins (TRL) during the postprandial period, thus prolonging postprandial lipemia in subjects with the TT genotype (19). Postprandial lipemia affects a number of steps in lipoprotein metabolism that may mediate effects on LDL susceptibility to oxidation in addition to those related to variations in plasma TRL concentration. Thus, the increased plasma TRL concentration during postprandial phase, causes cholesterol exchange between TRL and LDL and HDL mediated by CETP. In

postprandial state, it is postulated that the extent of exchange may be determined by particle residence time in the circulation (38). This implies an enchanced exchange in subjects with prolonged postprandial lipemia, as in TT subjects. The resultant triacylglycerol enriched LDL and HDL particles are subject to lipolysis by hepatic lipase, thus forming small, dense particles which are more susceptible to lipid peroxidation and therefore more atherogenics (39). These facts could partially explain the mechanisms via which the -219T allele of the *apoE* gene enhances LDL oxidation susceptibility.

LDL susceptibility to oxidation is determined by the content of antioxidant, the fatty acid composition and the size of the particle. As previously, we observed that the shift from a saturated fat-rich diet, or a diet rich in carbohydrates, to a monounsaturated-rich diet increased the resistance of LDL particles to oxidation (40). MUFA rich diets contain variable amounts of antioxidant micronutrients and phytochemicals, which may beneficially increase LDL oxidation resistance beyond their fatty acid composition (41).The mechanism that could take place in the increased oxidative stress of LDL when subjects are on a saturated fat diet, given these fatty acids are not prone to oxidation, probably involves other factors as the LDL concentration and the particle residence time in the circulation. Thus, SFA diet was associated with significant higher LDL-C plasma concentration as compared with both hypolipidemic diets. Furthermore we have observed a correlation between the increase in LDL cholesterol and the decrease in lag time.

Our data seem to indicate that *apoE* gene promoter (-219G/T) polymorphism influences lipoprotein levels and LDL susceptibility to oxidation according to the presence of saturated fatty acid in the diet of healthy young normolipemic men. We only included healthy young normolipemic men in order to avoid the effect of other factors (age, sex, BMI, etc) on lipid response to the content and quality of dietary fat. Studies conducted with conditions representing a impaired metabolism, as dyslipoproteinemic subjects, will generally be more

successful in finding differential effects across *apoE* gene promoter (-219G/T) genotypes, and such studies may be helpful in the future to clarify apoE gene-nutrition relationships. In contrast, the results obtained in this study suggest the use of genotyping of *apoE* gene promoter (-219G/T) polymorphism in the design of more precise dietary counselling and intervention and more efficacious primary and secondary CHD prevention.

In conclusion, healthy normolipidemic men carriers of the T allele are more susceptible to the presence of SFA in diet because of a greater increase in ApoB, LDL cholesterol concentrations and oxidative modifications in LDL. The allelic variability in the apoE (-219G/T) gene promoter polymorphism could partially explain differences in individual response to diet.

ACKNOWLEDGMENTS

JAM was responsible for the collection of data, analysis of data, and writing of the manuscript. CM and PG contributed to the collection and analysis of data. PPM provided statistical advice and contributed to the writing of the manuscript. CB and RM contributed to the collection of data and the writing of the manuscript. FF was responsible for design of the study and analysis of data. FPJ and JLM were responsible for the conception and design of the study, analysis of data, and writing of the manuscript. None of the authors had any conflict of interest.

REFERENCES

1. Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. Nature.1989; 341:162-4.

2. Weisgraber, K.H. Apolipoprotein E: structure-function relationships. Adv Protein Chem. 1994; 45: 249-302. Review.

3. Miettinen TA, Gylling H, Vanhanen H, Ollus A. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during fat intake in men with different apoprotein E phenotypes. Arterioscler Thromb. 1992; 12:1044-1052.

4. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science. 1988; 240:622-630.

5. Mahley RW, Huang Y. Apolipoprotein E: from atherosclerosis to Alzheimer`s disease and beyond. Curr Opin Lipidol. 1999; 10: 207-217.

6. Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat Genet. 1996; 14: 55-61.

7. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell. 1992; 71: 343-53.

8. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. Arterioscler Thromb. 1994; 14: 605-16.

9. Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. Nat Med. 1998; 4: 1189-92.

10. Rubin J and Berglund L. Apolipoprotein E and diets: a case of gene-nutrient interaction? Curr Opin Lipidol. 2002; 13:25-32.

11. Talmud PJ, Waterworth DM. In-vivo and in-vitro nutrient-gene interactions. Curr Opin Lipidol 2000; 11:31-36.

12. Ordovas JM, Schaefer EJ. Genes, variation of cholesterol and fat intake and serum lipids. Curr Opin Lipidol. 1999; 10:15-22.

13. Ordovas JM. The genetics of serum lipid responsiveness to dietary interventions. Proc Nutr Soc 1999; 58:171-187.

14. Weggemans RM, Zock PL, Ordovas JM, et al. ApoE genotype and the response of serum cholesterol to dietary fat, cholesterol and cafestol. Atherosclerosis. 2001; 154:547-555.

15. Artiga MJ, Bullido MJ, Sastre I, Recuero M, Garcia MA, Aldudo J, Vazquez J, Valdivieso F. Allelic polymorphisms in the transcriptional regulatory region of apolipoprotein E gene. FEBS Lett. 1998; 421:105-8

16. Lambert JC, Pasquier F, Cottel D, Frigard B, Amouyel P, Chartier-Harlin MC. A new polymorphism in the APOE promoter associated with risk of developing Alzheimer's disease. Hum Mol Genet. 1998; 7: 533-40.

17. Lambert JC, Brousseau T, Defosse V, Evans A, Arveiler D, Ruidavets JB, Haas B, Cambou JP, Luc G, Ducimetiere P, Cambien F, Chartier-Harlin MC, Amouyel P. Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations -the ECTIM Study. Hum Mol Genet. 2000; 9: 57-61.

18. Viitanen L, Pihlajamaki J, Miettinen R, Karkkainen P, Vauhkonen I, Halonen P, Kareinen A, Lehto S, Laakso M..Apolipoprotein E gene promoter (-219G/T) polymorphism is associated with premature coronary heart disease.J Mol Med. 2001; 79:732-7.

19. Moreno JA, Lopez-Miranda J, Marin C, Gomez P, Perez-Martinez P, Fuentes F, Fernandez De La Puebla RA, Paniagua JA, Ordovas JM, Perez-Jimenez F. The influence of the apolipoprotein E gene promoter (-219G/T) polymorphism on postprandial lipoprotein metabolism. J Lipid Res. 2003; 44:2059-64.

20. Human Nutrition Information Service, Department of Agriculture Composition of foods. Agriculture handbook no 8. Washington, DC: US Goverment Printing Office. 1987.

21. Varela G. Food composition tables (Tablas de composición de alimentos). Madrid: Instituto de Nutrición. CSIC. 1980.

22. Association of Official Analytical Chemists (1990) Official methods of analysis, 15th edn (modified). Association of Official Analytical Chemists, Arlington.

23. Ruiz-Gutiérrez V, Prada JL, Pérez-Jiménez F. Determination of fatty acid and triacylglycerol composition of human very-low-density lipoproteins. J Chromatogr. 1993; 622: 117-134.

24. Allain CC, Poon LS, Chang CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974; 20: 470-475.

25. Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem. 1973; 19: 476-482.

26. Assmann G, Schierwer H, Schmitz G, Hägele E. Quantification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid-$MgCl_2$. Clin Chem. 1983; 29: 2026-2030.

27. Riepponem P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-I and B in serum. Scand J Clin Lab Invest. 1987; 47: 739-744.

28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of a preparative ultracentrifugue. Clin Chem. 1972; 18: 499-502.

29. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun. 1989; 6: 67-75.

30. Tsukamoto K, Watanab T, Matsushima T, Kinoshita M, Kato H, Hashimoto Y, Kurokawa K, Teramoto T. Determination by PCR-RFLP of apo E genotype in a Japanese population. J Lab Clin Med. 1993; 121: 598-602.

31. Lopez Miranda, Jansen S, Ordovas JM, Salas J, Marin C, Castro P, Perez-Jimenez F. Infuence of the SstI polymorphism at the apolipoprotein C-III gene locus on the plasma low-density- lipoprotein- cholesterol response to dietary monounsaturated fat. Am J Clin Nutr. 1997; 66: 97-103.

32. Lopez-Miranda J, Ordovas JM, Espino A, Marin C, Salas J, Lopez Segura F, Perez-Jimenez F. Human apolipoprotein A-I gene promoter mutation predicts plasma low density lipoprotein cholesterol response to dietary fat in young males. Lancet. 1994; 343:1246-1249.

33. Lopez Miranda J, Ordovas JM, Mata P, Lichtenstein AH, Clevidence B, Judd JT. Effect of apolipoprotein E phenotype on diet induced plasma low density lipoprotein cholesterol lowering. J Lipid Res. 1994;35:1965-1975.

34. Shimano H, Yamada N, Katsuki M, Yamamoto K, Gotoda T, Harada K, Shimada M, Yazaki Y. Plasma lipoprotein metabolism in transgenic mice overexpressing apolipoprotein E. Accelerated clearance of lipoproteins containing apolipoprotein B. J Clin Invest. 1992; 90: 2084-91.

35. Davignon J, Cohn JS, Mabile L, Bernier L. Apolipoprotein E and atherosclerosis : insight from animal and human studies. Clin Chim Acta. 1999; 286:115-143.

36. Aviram M, Maor I, Keidar S, Hayek T, Oiknine J, Bar-El Y, Adler Z, Kertzman V, Milo S. Lesioned low density lipoprotein in atherosclerotic apolipoprotein E-deficient transgenic mice and in humans is oxidized and aggregated. Biochem Biophys Res Commun. 1995; 216: 501-13.

37. Ferré N, Camps J, Paul A, Cabré M, Calleja L, Osada J, Joven J. Effects of high-fat, low-cholesterol diets on hepatic lipid peroxidation and antioxidants in apolipoprotein E-deficient mice. Mol Cell Biochem. 2001; 218: 165-169.

38. Mann CJ, Yen FT, Grant AM, Bihain BE. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest. 1991; 88:2059-66.

39. Lechleitner M, Hoppichler F, Foger B, Patsch JR. Low-density lipoproteins of the postprandial state induce cellular cholesteryl ester accumulation in macrophages. Arterioscler Thromb Vasc Biol 1994; 14:1799-1807.

40. Lopez-Miranda J, Gomez P, Castro P, Marin C, Paz E, Bravo MD, Blanco J, Jimenez-Pereperez J, Fuentes F, Perez-Jimenez F. Mediterranean diet improves low density lipoprotein susceptibility to oxidative modifications. Med Clin (Barc). 2000; 115: 361-5.

41. Visioli F, Galli C. Antiatherogenic components of olive oil. Curr Atheroscler Rep 2001; 3: 64-7.

TABLE 1. Baseline characteristics of plasma lipids and apolipoproteins according to the -219G/T *apoE* promoter polymorphism.

	GG (n=7)	**GT** (n=38)	**TT** (n=10)
Age (years)	20.6±1.6	21.4±1.8	20.8±2.2
BMI (Kg/m^2)	24.3±2.4	23.4±2.7	23.8±2.9
Total-C (mmol/L)	4.1±0.5	4.1±0.7	4.1±0.6
LDL-C (mmol/L)	2.4±0.3	2.5±0.6	2.4±0.5
HDL-C (mmol/L)	1.2±0.2	1.18±0.26	1.3±0.4
Apo A-1 (g/L)	1.2±0.2	1.2±0.2	1.2±0.2
Apo B (g/L)	0.5±0.1	0.6±0.1	0.6±0.2
Triacylglycerol (mmol/L)	1.0±0.4	0.8±0.5	0.9±0.4

Values are mean ± SD. ANOVA. There were no significant differences between genotypes.

TABLE 2. Daily intake during each experimental diet period.

	SFA Diet	CHO Diet	MUFA Diet
Protein (% of energy intake)			
Calculated	15	15	15
Analyzed	18.0	17.5	17.7
Fat (% of energy intake)			
Saturated			
Calculated	20	10	10
Analyzed	22.2	9.1	9.1
Monounsaturated			
Calculated	12	12	22
Analyzed	11.1	13.2	24.1
Polyunsaturated			
Calculated	6	6	6
Analyzed	5.1	5.2	4.9
Carbohydrates (% of energy intake)			
Calculated	47	57	47
Analyzed	44.2	54.5	44.1
Complex	27.1	33.3	27.5
Simple	17.1	21.2	16.6
Cholesterol (mg/d)			
Calculated	285	285	285
Analyzed	272	275	277
Fiber (g/d)			
Calculated	30	30	30
Analyzed	25.8	26.0	24.6
Energy (MJ)	10.2	10.2	10.2

SFA, saturated fatty acids-rich diet; CHO, low-fat, high-carbohydrate diet; MUFA, monounsaturated fatty acids-rich diet.

TABLE 3.

Plasma Lipids (in mmol/L) and Apoproteins (in g/L) at the end of each dietary period according to genotype and the percentage change in LDL-cholesterol, ApoB and Lag times between saturated fatty acid-rich (SFA) and low-fat, high-carbohydrate (CHO) and between the SFA and monounsaturated fatty acid-rich (MUFA) diets by genotype.

Genotype-Diet	Lag time	TC	LDL-C	HDL-C	TG	Apo A-I	Apo B
GG (n =7)							
SFA	67.75±40.48 [a]	3.87±0.41	2.23±0.38 [a]	1.18±0.19	0.95±0.48	1.22±0.20	0.54±0.11 [a]
CHO	77.25±46.16 (14%)	3.61±0.49	2.09±0.38 (-5%)	1.06±0.22	0.95±0.24	1.11±0.17	0.52±0.13 (-5%)
MUFA	84.00±37.91 (30%)	3.59±0.48	2.06±0.44 (-7%)	1.08±0.19	0.94±0.20	1.14±0.15	0.51±0.11 (-5%)
GT (n = 38)							
SFA	45.58±20.88 [a]	4.30±0.67	2.68±0.66 [b]	1.20±0.31	0.88±0.32	1.25±0.22	0.67±0.17 [b]
CHO	47.44±26.08 (12%)	3.70±0.60	2.20±0.55 (-17%)	1.08±0.21	0.86±0.37	1.16±0.20	0.56±0.14 (-16%)
MUFA	54.91±24.05 (34%)	3.75±0.63	2.22±0.61 (-17%)	1.14±0.27	0.81±0.26	1.20±0.23	0.57±0.14 (-14%)
TT (n = 10)							
SFA	25.25±14.22 [b]	4.35±0.45	2.80±0.47 [b]	1.13±0.26	0.87±0.40	1.19±0.18	0.67±0.15 [b]
CHO	39.37±18.58 (72%)	3.77±0.46	2.23±0.57 (-21%)	1.13±0.36	0.84±0.38	1.15±0.18	0.59±0.19 (-14%)
MUFA	54.50±15.94 (154%)	3.95±0.35	2.33±0.42 (-16%)	1.23±0.37	0.82±0.34	1.22±0.22	0.60±0.18 (-11%)
P							
Diet	0.001	0.001	0.001	0.032	0.695	0.002	0.001
Genotype	0.025	0.485	0.459	0.912	0.692	0.831	0.371
Interaction	0.032	0.057	0.048	0.005	0.838	0.221	0.038

(Means ± SD), percentage change from SFA in brackets; TC, total cholesterol; TG, triacylglycerol. Different superscript letters indicate significant differences between genotypes groups for a given diet group, $P<0.05$ (repeated-measures ANOVA).