1   **The effect of dietary fat on low-density-lipoprotein size is influenced by apolipoprotein E**

2   **genotype in healthy subjects.**

3

9

10   Juan Antonio Moreno, Francisco Pérez-Jiménez, Carmen Marín, Purificación Gómez, Pablo Pérez-

11   Martínez, Rafael Moreno, Cecilia Bellido, Francisco Fuentes, José López-Miranda.

12

13   Lipids and Atherosclerosis Research Unit. Hospital Universitario Reina Sofía. Córdoba. Spain.

14

15   **Correspondence and reprint requests to**: José López-Miranda. Unidad de Lípidos y

16   Arteriosclerosis. Hospital Universitario Reina Sofía. Avda Menéndez Pidal, s/n. 14004 Córdoba,

17   Spain. Phone: 34-957-218250 FAX: 34-957-218250

18   **E-mail**: md1lomij@uco.es

19

20   **Running title:** apoE genotype, LDL size and dietary fat content.

21

22   **Words of the entire manuscript:** 5648

23   **Number of figures:** 0

24   **Number of tables:** 5

25 **ABSTRACT**

26

27   LDL particle size is dependent on both genetic factors, and environmental factors such as dietary

28   fat composition. apolipoprotein E (apoE) genotype is a major genetic determinant of LDL size.

29   Thus, the aim of this work was to study whether the *apoE* genotype interacts with the quantity and

30   quality of dietary fat modifying LDL size in young healthy subjects. 84 healthy subjects (66 apoE

31   3/3, 8 apoE 4/3, 10 apoE 3/2) were subjected to three dietary periods, each lasting four weeks.

32   The first was a saturated fat (SFA) enriched diet (38% fat, 20% SFA), which was followed by a

33   carbohydrate (CHO)-rich diet (30% fat, <10% SFA, 55% carbohydrate) or a monounsaturated

34   fatty acid (MUFA) olive oil-rich diet (38% fat, 22% MUFA) following a randomized crossover

35   design. At the end of each diet period, LDL particle size and plasma levels of total cholesterol,

36   LDL-C, HDL-C, apoB, apoA-I and triacylglycerols were determined. LDL particle size was

37   significantly higher ($p < 0.04$) in subjects with the apoE 4/3 genotype compared to apoE 3/3 and

38   apoE 3/2 in basal state. LDL size was smaller ($p < 0.02$) after CHO diet than after MUFA or SFA

39   diets. After CHO diet, a significant increase in LDL particle size ($p < 0.035$) was noted with

40   respect to MUFA diet in the apoE 4/3 subjects, whereas a significant decrease was observed in

41   the apoE 3/3 individuals ($p < 0.043$). In conclusion, a Mediterranean diet, high in MUFA-fat

42   increases LDL particle size in comparison to a CHO diet and this effect is dependent of apoE

43   genotypes.

44

45   **Key words:** *apoE* gene polymorphism; diet; LDL size.

46

47

48   Apolipoprotein E (ApoE) plays an important role in lipid metabolism both promoting

49 efficient uptake of triglyceride-rich lipoproteins from the circulation (1,2) and taking part in the

50 cellular cholesterol efflux and reverse cholesterol transport (3). However, such functions are not

51 uniformly effective because ApoE is present in the population in three main isoforms (ApoE2,

52 ApoE3 and ApoE4). These proteins determine changes in ApoE plasma concentrations and differ

53 in their affinity to its specific receptors (4,5).

54   The apoE4 variant has been associated with an increased low-density lipoprotein (LDL)

55 production from very low-density lipoprotein (VLDL), increased uptake of postprandial

56 lipoproteins, increased intestinal absorption of cholesterol, decreased bile acid synthesis and

57 faster LDL clearance from plasma compared to apoE3 or apoE2 variants (6-8). The apoE2 allele

58 has been consistently associated with lower LDL-cholesterol and ApoB levels and higher

59 triacylglycerol, HDL-cholesterol and apoAI concentrations (9-11).

60   The highest ApoB, triacylglycerol, total and LDL-cholesterol levels associated with the

61 apoE4 isoform (12-15) are related with the intake of diets enriched in saturated fat and

62 cholesterol (16,17). These findings have caused that the interaction between lipoprotein

63 responsiveness to dietary manipulation and apoE alleles has been the subject of several studies.

64 However the results have been controversial (18). Whereas some studies have found a

65 pronounced dietary responsiveness for apoE4 carriers, others have reported no difference in

66 response across apoE genotypes to changes in dietary fat or cholesterol content (19-21). Thus,

67 the hyperresponse of LDL cholesterol concentrations associated with the E4 allele occurred only

68 when the fat content in diet varied (22).

69   Several studies suggest that the presence of small, dense LDL particles is associated with

70 an increased risk of coronary artery disease (CAD) (23). LDL particle size is dependent on both

71 genetic factors and environmental factors such as dietary fat composition. Low-fat, high-

72   carbohydrate diets decreased mean LDL size compared with high saturated fat diets (24-25); the

73   largest and smallest subfractions decreased in concentration, whereas the intermediate-small

74   fraction increased. Monounsaturated fat diets, slightly reduced (26) or did not affect (27) LDL

75   size compared with saturated fat diets. Overall, it is difficult to provide a clinical interpretation to

76   infer benefit or harm from such changes in LDL sizes during these interventions. On the other

77   hand, several authors have indicated that the apoE genotype is a major genetic determinant of

78   LDL size although results are contradictory. While some data show that subjects carrying apoE2

79   allele have smaller and denser LDL compared with subjects carrying the apoE4 allele (28), other

80   studies failed to show this relationship (29) or even that subjects carrying apoE4 allele have

81   smaller LDL particle diameter than subjects with the apoE2 allele (15,30). Interestingly, even

82   though it is well known that both apoE phenotype and diet modify LDL size, studies showing the

83   interaction between these two factors are scarce or discrepant. Thus, a higher saturated fat intake

84   was associated with smaller LDL particles in apoE2 subjects, and larger LDL particles in apoE4

85   subjects (31). However, other study showed that when subjects changed from a high-fat to a low-

86   fat diet there was a shift from large buoyant cholesterol-rich particles, to smaller denser LDL

87   particles, with progressively greater reductions in levels of larger LDL from apoE3/2 to apoE3/3

88   to apoE3/4 (32). Therefore, the aim of this work was to study whether the apoE genotype

89   interacts with the quantity and quality of dietary fat modifying LDL size in young healthy

90   subjects.

91

92

93

94

95

96   **MATERIALS AND METHODS**

97

98   **Human Subjects**

99   A group of 84 subjects (66 apoE 3/3, 8 apoE 4/3, 10 apoE 3/2), including both men

100   (n=58; 4/3=4, 3/3=46 and 3/2=8) and women (n=26; 4/3=4, 3/3·=20 and 3/2=2), were recruited

101   from among students at the University of Cordoba. The subjects had a mean age ($\pm$ SD) of 21.55

102   $\pm$ 0.40 years. Informed consent was obtained from all participants. All subjects underwent a

103   comprehensive medical history, physical examination, and clinical chemistry analysis before

104   enrolment. Subjects showed no evidence of any chronic disease (hepatic, renal, thyroid, or

105   cardiac dysfunction), obesity or unusually high levels of physical activity (e.g. sports training).

106   None of the subjects had a family history of premature coronary artery disease or had taken

107   medications or vitamin supplements in the 6 months prior to the study. Physical activity and diet,

108   including alcohol consumption, were recorded in a personal log for 1 week and the data were

109   used to calculate individual energy requirements. Mean body mass index (BMI) was 22.86 $\pm$ 0.28

110   Kg/m^2 (mean $\pm$ SD) at the onset of the study and remained constant throughout the experimental

111   period. Subjects were encouraged to maintain their regular physical activity and lifestyle and

112   were asked to record in a diary any event that could affect the outcome of the study, such as

113   stress, change in smoking habits and alcohol consumption or intake of foods not included in the

114   experiment design. The study protocol was approved by the Human Investigation Review

115   Committee at the Reina Sofia University Hospital.

116

117   **Diets**

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

119 saturated fatty acid (SFA)-rich diet, with 15% protein, 47% carbohydrate and 38% fat (20%

120 SFA, 12% monounsaturated fatty acid (MUFA) and 6% polyunsaturated fatty acid (PUFA)).

121 After this period, volunteers were randomly assigned to 1 of 2 diet sequences. 42 subjects

122 received a MUFA-rich diet containing 15% protein, 47% carbohydrates and 38% fat (<10%

123 SFA, 6% PUFA, 22% MUFA) for 28 days. This diet was followed for 28 days by consumption

124 of a carbohydrate (CHO)-rich diet containing 15% protein, 55% carbohydrates and <30% fat

125 (<10% SFA, 6% PUFA, 12% MUFA). The other 42 subjects consumed the CHO diet before the

126 MUFA diet. The cholesterol content remained constant (under 300 mg/day) during the three

127 periods. 80% of the MUFA diet was provided by virgin olive oil, which was used for cooking,

128 salad dressing and as a spread. Carbohydrate intake of the CHO diet was based on the

129 consumption of biscuits, jam and bread. Butter and palm oil were used during the SFA dietary

130 period.

131   The composition of the experimental diets was calculated using the United States

132 Department of Agriculture (33) food tables and Spanish food composition tables for local

133 foodstuffs (34). All meals were prepared in the hospital kitchen and were supervised by a

134 dietitian. Lunch and dinner were eaten in the hospital dining room, whereas breakfast and an

135 afternoon snack were eaten in the medical school cafeteria. Fourteen menus were prepared with

136 regular solid foods and rotated during the experimental period. Duplicate samples from each

137 menu were collected, homogenized, and stored at –70ºC. Protein, fat, and carbohydrate contents

138 of the diet were analysed by standard methods (35). Dietary compliance was verified by

139 analysing the fatty acids in LDL cholesterol esters at the end of each dietary period (36). The

140 study took place from January to March to minimize seasonal effects and academic stress.

141

142 **Lipid analysis, biochemical determinations and LDL size.**

143   Venous blood samples were collected into EDTA-containing (1g/L) tubes from all

144   subjects after a 12-h overnight fast at the beginning of the study and at the end of each dietary

145   period. Plasma was obtained by low speed centrifugation for 15 min at 4ºC within 1h of

146   venipuncture. To reduce interassay variation, plasma was stored at -80ºC and analysed at the end

147   of the study. Plasma total cholesterol (TC) and triacylglycerol levels were determined by

148   enzymatic techniques (37,38). HDL cholesterol was determined after precipitation with

149   fosfowolframic acid (39). Apo A-I and Apo B were determined by immunoturbidimetry (40).

150   LDL cholesterol concentration was calculated using the Friedewald formula (41). The

151   predominant LDL peak particle diameter (LDL size) was measured from serum samples with

152   polyacrylamide gradient gel electrophoresis as described (42). We identified two subclass LDL-

153   phenotypes using the criteria described previously (43): the classical category, phenotype A, is

154   more than 25.5 nm, and phenotype B is 25.5 nm or less.

155

156   **DNA amplification and genotyping of ApoE**

157   Amplification of a region of 266-bp of the apoE gen was done by PCR with 250 ng of

158   genomic   DNA   and   0.2   μmol   of   each   oligonucleotide   primer   (E1,   5'-

159   GAACAACTGACCCCGGTGGCGGAG-3',   and   E2,   5'-

160   TCGCGGGCCCCGGCCTGGTACACTGCCA-3') and 10% dimethyl sulfoxide in 50 μl. DNA

161   was denatured at 95 ºC for 5 min followed by 30 cycles of denaturation at 95 ºC for 1 min,

162   annealing at 63 ºC for 1.5 min, and extension at 72 ºC for 2 min. Twenty μl of the PCR product

163   were digested with 10 units of restriction enzyme *CfoI* (BRL, Maryland, USA) in a total volume

164   of 35 μl. Digested DNA was separated by electrophoresis on an 8% non-denaturing

165   polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

166

167 **Statistical analysis**

168   We used the analysis of variance (ANOVA) for repeated measures to test for effects of

169 the apoE gene polymorphism on plasma total cholesterol, LDL cholesterol, HDL cholesterol,

170 trialcylglycerol, Apo A-I, and Apo B concentration and LDL size in each dietary stage.

171 Triacylgycerols levels were log transformer prior to statistical analyses. When statistical

172 significance was found, Tukey's post hoc comparison test was used to identify group differences.

173 Chi-square test was used to determine whether the replacement of high-fat diets (SFA or MUFA)

174 by low-fat, high carbohydrate diet (CHO) increases the number of subjects with LDL-phenotype

175 B. Statistical significance was considered at $p < 0.05$. Statistical analyses were carried out using

176 the SPSS statistical software, version 8.0 (SPSS Inc, Chicago).

177

178 **RESULTS**

179

180   Significant differences were observed when we compared basal characteristics according

181 to *apoE* genotypes (**Table 1**). Thus LDL particle size and total cholesterol (TC), LDL

182 cholesterol, and ApoB plasma concentrations were higher in subjects with the apoE 4/3 genotype

183 compared to apoE 3/3 and apoE 3/2 subjects. The actual composition of the mean daily intake of

184 the participants is shown in **Table 2**. Analysis of LDL-cholesterol esters obtained after each

185 dietary period showed good adherence in the different intervention stages. After the SFA-diet

186 period, we observed a significantly greater ($p<0.005$) increase in palmitic acid in the LDL-

187 cholesterol esters than were observed after the CHO and MUFA diets: 27.3 ± 1.4 % compared

188 with 19.8 ± 3.9 % and 15.2 ± 0.4 %, respectively. A significantly greater ($p<0.05$) increase in

189 oleic acid in the cholesterol esters was also seen after the MUFA diet (50.3 ± 4.7 %) than after

190 the CHO diet (38.8 ± 9.0 %), but not after the SFA diet (47.2 ± 4.4 %).

191   Dietary intervention had significant effects on LDL particle size and plasma TC, LDL-C,

192 HDL-C, ApoA-I and ApoB concentrations (**Table 3**), but failed to exert statistically significant

193 changes on triacylglycerol plasma concentration. In comparison with the SFA diet, the CHO diet

194 was associated with a decrease in the plasma TC (-0.56 mmol/L, $p<0.001$), LDL-C (-0.44

195 mmol/L, $p<0.001$), HDL-C (-0.11 mmol/L, $p<0.001$), ApoA-I (-0.10 g/l $p<0.001$), and ApoB (-

196 0.07 g/L, $p<0.002$) concentrations. The MUFA diet also showed similar effects, with decreases in

197 the concentrations of TC (-0.49 mmol/L, $p<0.001$), LDL-C (-0.41 mmol/L, $p<0.001$), HDL-C (-

198 0.05 mmol/L, $p<0.001$), ApoA-I (-0.06 g/l $p<0.001$) and ApoB (-0.08 g/L, $p<0.002$). However,

199 in comparison with the MUFA diet, the CHO diet was associated with a decrease in the plasma

200   concentrations of HDL-C (-0.06 mmol/L, p<0.001) and apoA-I (-0.04 g/l p<0.001). LDL particle

201   size was lower (p<0.02) after the CHO diet than after the high MUFA diet and high SAT diet.

202   LDL-size was negatively correlated with triacylglycerols levels (r=-0.234; p<0.0009). Women

203   had significant (p<0.05) higher LDL particle size and higher HDL-C and ApoA-I plasma

204   concentration compared to men independently of the quality and quantity of dietary fat (data not

205   shown). The shift from SFA and MUFA diets to CHO diet increases significantly (p<0.0001) the

206   number of subjects with smaller LDL particle diameters (phenotype B). The increase in number

207   of subjects with phenotype B after CHO diet was observed in both men and women (**Table 4**).

208   The *apoE* genotype has a significant effects on ApoB (p=0.003), total cholesterol

209   (p=0.032) and LDL-cholesterol (p=0.023) levels. Thus, ApoB, total and LDL cholesterol plasma

210   concentrations were higher in subjects with the apoE 4/3 genotype, intermediate in apoE 3/3

211   subjects, and lower in apoE 3/2 subjects after the CHO, SFA and MUFA diets (**Table 5**).

212   Significant differences (p<0.009) in LDL-cholesterol levels were observed between apo4/3 vs

213   apo3/3 and apo3/2 subjects when changing their diet from MUFA to CHO (**Table 5**). Subjects

214   with apoE 4/3 genotype had an increase of 0.31±0.19 mmol/L after changing their diet from

215   MUFA to CHO whereas subjects with apoE 3/3 maintained with almost similar levels of LDL-C

216   and subjects with apoE 3/2 had a decrease of 0.15 mmol/L. LDL-size was larger (p<0.05) in

217   apoE 4/3 subjects than in apoE 3/3 and apoE 3/2 subjects after SFA, CHO and MUFA diets. In

218   addition there was a decrease in LDL-size in subjects with apoE 4/3 after changing from a CHO

219   diet to a MUFA diet (0.22 ± 0.15, p<0.035) while in subjects with apoE 3/3 there was an increase

220   (0.17 ± 0.06, p<0.043). However, there was not significant interaction between gender and LDL

221   size by genotype.

222

223

224 **DISCUSSION**

225

226   Our results show that replacement from a CHO diet to a MUFA diet increases the LDL-

227 size in apoE 3/3 young healthy subjects while decreasing LDL-size in apoE 4/3 subjects.

228   A diet high in saturated fat contributes to the development of CAD, so dietary

229 intervention is recommended to lower plasma lipid levels. However, it is not clear whether

230 saturated fat should be replaced by carbohydrates or monounsaturated fat. In accordance with our

231 results, previous studies indicate that both MUFA and CHO diets reduce total and LDL

232 cholesterol (44,45). However when subjects consumed a MUFA diet the levels of HDL

233 cholesterol and ApoA-I are higher as compared to a CHO diet. In addition we observed that low-

234 fat, high-carbohydrate diets compared with high saturated fat decrease mean LDL size

235 (24,25,31,32). Furthermore, our data confirm the results obtained in a recent study where no

236 differences in LDL size were observed between MUFA and SFA diet (27). But more importantly,

237 our study shows that a Mediterranean diet enriched in monounsaturated fatty acids, increases

238 LDL size in comparison with a low-fat, high-carbohydrate diet. Studies employing analysis of

239 LDL subclasses have demonstrated heterogeneity of the LDL response to low fat, high

240 carbohydrate diets in healthy subjects (46). In individuals with a genetically influenced

241 atherogenic lipoprotein phenotype, characterized by a predominance of small dense LDL

242 (phenotype B), lowering of plasma LDL cholesterol levels by low fat diets has been found to

243 represent a reduction in numbers of circulating small LDL particles. In contrast, in the majority

244 of healthy individuals with larger LDL (phenotype A), a significant proportion of the low fat diet-

245 induced reduction in plasma LDL cholesterol is made by depletion of the cholesterol content of

246 LDL particles and the shift from larger to smaller LDL particle diameters. Moreover, with

247 progressive reduction of dietary fat and isocaloric substitution of carbohydrate, an increasing

248   number of subjects with phenotype A convert to the phenotype B, as we have observed.

249   One of the most intriguing aspects of the response of plasma cholesterol to diet is how it

250   varies among individuals. The influence of the genetic loci of the principal apolipoproteins such

251   as the AI-CIII-AIV complex and the apoE gene has been studied (22,47,48). In agreement with

252   previous studies (9-10), we have observed that the presence of the apoE4 allele has been

253   associated with a proatherogenic lipoprotein profile, with increased ApoB, total and LDL

254   cholesterol plasma concentrations. The effect of the apoE genotypes on plasma lipids is modified

255   by environmental factors such as dietary intake (12,49). Thus the hyperresponse of LDL

256   cholesterol concentrations associated with the E4 allele occurred only when the fat content in diet

257   varied (23). We have observed that replacement from a CHO diet to a MUFA diet was associated

258   with a significant decrease of LDL-C in apoE 3/4 subjects, whereas the opposite or not effect was

259   observed for apoE 3/2 and apoE 3/3 subjects respectively.

260   The relationship between apoE genotype and LDL size have provided contrasting results

261   (15,26,28-31,50). In accordance with previous studies, we have observed that apoE2 subjects

262   have smaller LDL particles than the other groups (29,31,50) and changes in LDL size were

263   inversely correlated with triacylglycerol levels (28). The mechanism by which apoE isoforms

264   might affect LDL particle size is not completely clear. Barballo et al. (50) speculate that this

265   genetic response probably involves the effect of apoE2 on receptor-mediated VLDL clearance.

266   Thus, the decreased uptake of VLDL and VLDL remnants by the B/E receptor in apoE2 subjects

267   leads to higher levels of triglyceride-rich lipoproteins with an increased conversion into smaller

268   and denser LDL particles. However, other studies have reported that apoE2 subjects have similar

269   or even larger LDL particles in comparison with apoE3 and apoE4 individuals (15,30,31).

270   Environmental factors, such as diet or geographical differences, could explain the variability

271   observed in these studies. In agreement with this hypothesis, some studies have examined the

272 effect of apoE gene-diet interaction on LDL-size. Thus, Campos et al. (31) divided free-living

273 Costa Ricans according to their intake of saturated fat and observed that higher saturated fat

274 intake was associated with smaller LDL particles in apoE2 subjects, and larger LDL particles in

275 apoE4 subjects. However the gene-diet interaction was not statistically significant for LDL

276 particle size. This study was carried out in a population under normal daily conditions without

277 dietary intervention. In our study, subjects were randomly assigned to a dietary intervention

278 study, which means that the results obtained are more reliable. Thus, the replacement of a CHO

279 diet by a MUFA diet increases LDL-size in apoE 3/3 while decreasing it in apoE 4/3 subjects.

280 We also observed that LDL-size was larger in apoE 4/3 subjects than in apoE 3/3 and apoE 3/2

281 subjects after SFA, CHO and MUFA diets. However, Dreon et al. (32) found that reduction in

282 dietary fat resulted in a shift from large buoyant cholesterol-rich particles, to smaller denser LDL

283 particles, with progressively greater reductions in levels of larger LDL from apoE 3/2 to apoE 3/3

284 to apoE 3/4. Their results apply only to reduction in total fat intake, and it is possible that apoE

285 isoforms operate differently in influencing the response to other dietary manipulation, such as

286 substitution of monounsaturated fat or carbohydrates for saturated fat intake.

287 Studies in vitro have demonstrated that oleic acid is a potent stimulator of TRL

288 (Triacylglycerol rich lipoproteins) secretion (51), and test-meal studies have found that meals

289 high in oleic acid-rich oils result in a more pronounced and sharper postprandial rise in plasma

290 TRL compared with saturated fatty-acid-rich meals (52). The apoE4 variant has been associated

291 with increased uptake of postprandial lipoproteins compared to apoE3 or apoE2 variants (6).

292 Therefore, apoE4 subjects would have lower levels of TRL with a decreased conversion into

293 smaller and denser LDL particles (24). A MUFA diet, high in oleic acid from virgin olive oil,

294 could regulate the increased uptake of TRL in apoE4 subjects. This would explain the decreased

295 in LDL particle size observed in our study when apoE4 subjects changed from a CHO diet to

296   MUFA diet. We have only observed this effect in apoE4 subjects probably because carriers of the

297   apoE4 allele have a greater lipid response to dietary changes than individuals not possessing the

298   apoE4 allele (22). Although it is thought that increased intake of carbohydrates decreases LDL

299   size (24,25), it is not known whether reduced dietary fat intake contributes to these lipoprotein

300   changes to a lesser extent than the MUFA diet, as we have observed. New studies are needed,

301   therefore, to confirm our results. It is important to note that one of the limitations to genetic

302   association studies is the difficulty in corroborating findings observed in populations with

303   different characteristics. We must be cautious therefore when extrapolating the results to a more

304   general population

305   In conclusion, our data indicate that each subject needs to be examined and guided as to

306   diet individually. No diet can be recommended unequivocally without knowing more about those

307   who are targeted. Even though a MUFA-rich diet, increases LDL size in comparison with a

308   CHO-rich diet, this effect is dependent of apoE genotypes. Thus, the replacement of a CHO diet

309   by a MUFA diet increases LDL-size in apoE 3/3 while decreasing it in apoE 4/3 subjects.

310

311

312   **ABBREVIATIONS**

313

314   Abbreviations used: apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease,

315   CHO, carbohydrate; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid;

316   SFA, saturated fatty acid; TC, total cholesterol; TRL, triacylglycerol rich lipoproteins.

LITERATURE CITED

1. Beisiegel U, Weber W, Ihrke G, Herz J & Stanley KK. (1989). The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. Nature. 14:162-4.

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

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

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

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

6. Gylling H, Kontula K & Miettinen TA. (1995). Cholesterol absorption and metabolism and LDL kinetics in healthy men with different apoprotein E phenotypes and apoprotein B XbaI and LDL receptor PvuII genotypes. Arterioscler Thromb Vasc Biol. 15: 208-213.

7. Weintraub MS, Eisenberg S & Brelow JL. (1987). Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest. 80: 1571-1577.

8. Nikkilä M, Solakivi T, Lehtimäki T, Koivula T, Laippala P & Astrom B. (1994). Postprandial plasma lipoprotein changes in relation to apolipoprotein E phenotypes and low density lipoprotein size in men with and without coronary artery disease. Atherosclerosis. 106: 149-157.

9. Dallongeville J, Sussier-Cacan S & Davignon J. (1992). Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J Lipid Res. 33: 447-54.

10. Wilson PWF, Myers RH, Larson MG, Ordovas JM, Wolf PA & Schaefer EJ. (1994). Apolipoprotein E alleles, dislipidemia, and coronary heart disease: the Framingham offspring study. JAMA. 272:1666-71.

11. Kataoka S, Robbins DC, Cowan LD, Go O, Yeh JL, Devereus RB, Fabsitz RR, Lee ET, Welty TK & Howard BV. (1996). Apolipoprotein E polymorphism in American Indians and its relation to plasma lipoproteins and diabetes: the Strong Heart Study. Arterioscler Thromb Vasc Biol 16: 918-25.

12. Davignon J, Gregg RE & Sing CF. (1988). Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis. 8: 1-21.

13. Wilson PWF, Schaefer EJ & Larson MG. (1996). Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol. 16:1250-1255

14. Utermann G, Pruin N & Steinmetz A. (1979). Polymorphism of apolipoprotein E. Effect of a single polymorphic gene locus on plasma lipid levels in men. Clin Genet. 15: 63-72.

15. Schaefer EJ, Lamon-Fava S, Johnson S, Ordovas JM, Schaefer M, Castelli WP & Wilson PWF. (1994). Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Arterioscler Thromb. 14:1105–1113

16. Ehnholm C, Lukka M, Kuusi T, Nikkila E & Utermann G. (1986). Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J Lipid Res. 27: 227-235

17. Utermann G. (1987). Apolipoprotein E polymorphism in health and disease. Am Heart J. 113: 433-440.

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

19. Talmud PJ & Waterworth DM. (2000). In-vivo and in-vitro nutrient-gene interactions. Curr Opin Lipidol. 11: 31-36.

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

21. Weggemans RM, Zock PL, Ordovas JM, Pedro-Botet J & Katan MB. (2001). Apolipoprotein E genotype and the response of serum cholesterol to dietary fat, cholesterol and cafestol. Atherosclerosis. 154: 547-555.

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

23. Campos H, Genest JJ Jr, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, Wilson PW & Schaefer EJ. (1992). Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb. 12: 187-95.

24. Dreon DM, Fernstrom HA, Williams PT & Krauss RM. (1999). A very low-fat diet is not associated with improved lipoprotein profiles in men with a predominance of large, low-density lipoproteins. Am J Clin Nutr. 69: 411–418.

25. Krauss RM & Dreon DM. (1995). Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. Am J Clin Nutr. 629: 478-87.

26. Kratz M, Gulbahce E, von Eckardstein A, Cullen P, Cignarella A, Assmann G & Wahrburg U. (2002). Dietary mono- and polyunsaturated fatty acids similarly affect LDL size in healthy men and women. J Nutr. 132: 715–718.

27. Rivellese AA, Maffettone A, Vessby B, Uusitupa M, Hermansen K, Berglund L, Louheranta A, Meyer BJ & Riccardi G. (2003). Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. Atherosclerosis. 167: 149-58.

28. Dart AM & Cooper B. (1999). Independent effects of Apo E phenotype and plasma triglyceride on lipoprotein particle sizes in the fasting and postprandial states. Arterioscler Thromb Vasc Biol. 19: 2465-2473.

29. Zhao SP, Verhoeven MH, Vink J, Hollaar L, van der Laarse A, de Knijff P & van't Hooft FM. (1993). Relationship between apolipoprotein E and low density lipoprotein particle size. Atherosclerosis. 102: 147–154.

30. Haffner SM, Stern MP, Miettinen H, Robbins D & Howard BV. (1996). Apolipoprotein E polymorphism and LDL size in a biethnic population. Arterioscler Thromb Vasc Biol 16:1184–1188.

31. Campos H, D'Agostino M & Ordovas JM. (2001). Gene-diet interactions and plasma lipoproteins: role of apolipoprotein E and habitual saturated fat intake. Genet Epidemiol.20: 117-128.

32. Dreon DM, Fernstrom HA, Miller B & Krauss RM. (1995). Apolipoprotein E isoform phenotype and LDL subclass response to a reduce fat diet. Arterioscler Thromb Vasc Biol. 15: 105-111.

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

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

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

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

37. Allain CC, Poon LS, Chang CS, Richmond W & Fu PC. (1974). Enzymatic determination of total serum cholesterol. Clin Chem. 20:470-475

38. Bucolo G & David H. (1973). Quantitative determination of serum triglycerides by use of enzymes. Clin Chem.19:476-482.

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

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

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

42. Krauss RM & Burke DJ. (1982). Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res. 23: 97-104.

43. Austin MA & Krauss RM. (1986). Genetic control of low-density lipoprotein subclasses. Lancet. 2: 592-595.

44. Mensink RP & Katan MB. (1991). Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. Arterioscler Thromb. 12: 911-9.

45. Hegsted DM, Ausman LM, Johnson JA & Dallal GE. (1993). Dietary fat and serum lipids: an evaluation of the experimental data. Am J Clin Nutr. 57: 875-83.

46. Kraus RM. (2001). Atherogenic lipoprotein phenotype and diet-gene interaction. J Nutr. 131:

340-343.

47. Lopez-Miranda J, Jansen S, Ordovas JM, Salas J, Marin C, Castro P & Perez-Jimenez F. (1997). Influence 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. 66: 97-103.

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

49. Luc G, Bard JM, Arvellier D, Evans A, Cambou JP, Bingham A, Amouyel P, Schaffer P, Ruidavets JB, Cambien F, Fruckhart JC & Lucimetiere P. (1994). Impact of apolipoprotein E polymorphism on lipoproteins and risk of myocardial infarction: the ECTIM study. Arterioscler Thromb 14:1412-9.

50. Barbagallo CM, Polizzi F, Severino M, Rizzo M, Vivona N, Onorato F, Caldarella R, Cefalu AB, Noto D, Notarbartolo A & Averna MR. (2001). ApoE polymorphism in a small Mediterranean island: Relationships with plasma lipids, lipoproteins and LDL particle size. Eur J Epidem. 17:707-713

51. Black IL, Roche HM, Tully AM & Gibney MJ. (2002). Acute-on-chronic effects of fatty acids on intestinal triacylglycerol-rich lipoprotein metabolism. Br J Nutr. 88: 661-9.

52. Sanders TA, de Grassi T, Miller GJ & Morrissey JH. (2000). Influence of fatty acid chain length and cis/trans isomerization on postprandial lipemia and factor VII in healthy subjects (postprandial lipids and factor VII). Atherosclerosis. 149:413-20.

TABLE 1. Baseline characteristics of plasma lipids and apolipoproteins according to the *apoE* genotypes.

	ApoE 4/3	ApoE 3/3	ApoE 3/2
Age (years)	23.75±2.22	21.45±1.76	21.22±2.44
BMI (Kg/m^2)	22.23±2.79	22.69±3.24	23.02±3.89
Total-C (mmol/L)	4.58±0.52 [a]	4.09±0.67 [b]	3.78±0.49 [b]
LDL-C (mmol/L)	2.89±0.50 [a]	2.43±0.63 [b]	2.11±0.46 [b]
HDL-C (mmol/L)	1.37±0.36	1.28±0.35	1.18±0.34
Apo A-1 (g/L)	1.46±0.27	1.32±0.24	1.27±0.26
Apo B (g/L)	0.80±0.11 [a]	0.66±0.15 [b]	0.59±0.12 [b]
Triacylglycerol (mmol/L)	0.71±0.24	0.82±0.45	1.05±0.43
LDL size (nm)	26,34 ± 0.23 [a]	25.83 ± 0.08 [b]	25.70 ± 0.21 [b]

Values are mean ± SD. Different superscript letters indicate significant differences between genotypes. $P < 0.05$ (repeated-measures ANOVA).

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 lipid levels and LDL-size of the subjects after dietary intervention.

	SFA	CHO	MUFA
Total-Cholesterol (mmol/L)	4.24±0.62 [a]	3.68±0.60 [b]	3.75±0.64 [b]
LDL-C (mmol/L)	2.61±0.60 [a]	2.17±0.56 [b]	2.20±0.60 [b]
HDL-C (mmol/L)	1.23±0.29 [a]	1.12±0.25 [b]	1.18±0.29 [c]
Apo A-I (g/L)	1.35±0.24 [a]	1.25±0.22 [b]	1.29±0.24 [c]
Apo A-B (g/L)	0.68±0.15 [a]	0.59±0.15 [b]	0.60±0.15 [b]
Triacylglycerol (mmol/L)	0.82±0.37	0.82±0.35	0.79±0.31
LDL-Size (nm)	25.91±0.08 [a]	25.80±0.07 [b]	25.94±0.08 [b]

Values are mean ± SD. Different superscript letters indicate significant differences between genotypes. $P<0.001$ (repeated-measures ANOVA).

TABLE 4: Number of subjects with LDL phenotype A and phenotype B at the end of each dietary period.

	SFA	MUFA	CHO
Total population (n=84)			
A phenotype	67 (79.8%)[a]	68 (80.9%)[a]	41 (48.8%)[b]
B phenotype	17 (20.2%)[a]	16 (19.1%)[a]	43 (51.2%)[b]
Men (n=58)			
A phenotype	43 (74.1%)[a]	45 (77.6%)[a]	25 (43.1%)[b]
B phenotype	15 (25.9%)[a]	13 (22.4%)[a]	33 (56.9%)[b]
Women (n=26)			
A phenotype	23 (88.5%)[a]	23 (88.5%)[a]	16 (61.5%)[b]
B phenotype	3 (11.5%)[a]	3 (11.5%)[a]	10 (38.5%)[b]

Different superscript letters indicate significant differences between diets for a given LDL phenotype. $P < 0.05$. (Chi-square test).

TABLE 5: Plasma Lipids (in mmol/L), Apoproteins (in g/L) and LDL-size (in nm) at the end of each dietary period according to apoE genotype.

Genotype-Diet	TC	LDL-C	HDL-C	Apo A-I	Apo B	LDL-size
apoE 3/4 (n=8)						
SFA	4.48 ± 0.47^a	2.89 ± 0.34^a	1.28 ± 0.19	1.47 ± 0.24	0.80 ± 0.11^a	26.38 ± 0.54
CHO	4.21 ± 0.30^a	2.68 ± 0.25^a	1.17 ± 0.25	1.38 ± 0.22	0.76 ± 0.13^a	26.47 ± 0.68
MUFA	4.07 ± 0.47^a	2.37 ± 0.23^a	1.24 ± 0.22	1.39 ± 0.20	0.73 ± 0.07^a	26.26 ± 0.40
apoE 3/3 (n=66)						
SFA	4.22 ± 0.64^b	2.59 ± 0.62^b	1.24 ± 0.30	1.32 ± 0.23	0.67 ± 0.15^b	25.84 ± 0.08
CHO	3.66 ± 0.60^b	2.13 ± 0.56^b	1.13 ± 0.26	1.23 ± 0.21	0.58 ± 0.14^b	25.74 ± 0.09
MUFA	3.72 ± 0.61^b	2.18 ± 0.55^b	1.18 ± 0.30	1.27 ± 0.23	0.59 ± 0.14^b	25.91 ± 0.07
apoE 3/2 (n=10)						
SFA	3.92 ± 0.35^c	2.35 ± 0.04^c	1.14 ± 0.22	1.32 ± 0.20	0.58 ± 0.12^c	25.77 ± 0.34
CHO	3.30 ± 0.32^c	1.77 ± 0.24^c	1.03 ± 0.22	1.18 ± 0.19	0.50 ± 0.12^c	25.62 ± 0.12
MUFA	3.44 ± 0.53^c	1.92 ± 0.46^c	1.12 ± 0.26	1.22 ± 0.29	0.52 ± 0.15^c	25.69 ± 0.31
P						
Diet	0.001	0.001	0.001	0.001	0.001	0.001
Genotype	0.071	0.046	0.453	0.322	0.110	0.021
Interaction	0.032	0.023	0.517	0.219	0.003	0.035

Values are means ± SD. TC: total cholesterol. Different superscript letters indicate significant differences between genotypes groups for a given diet group, $P<0.05$ (repeated-measures ANOVA).