The Effect of Dietary Fat on LDL Size Is Influenced by Apolipoprotein E Genotype in Healthy Subjects[1]

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ABSTRACT LDL particle size is dependent on both genetic factors and environmental factors such as dietary fat composition. The apolipoprotein E (apoE) genotype is a major genetic determinant of LDL size. Thus, the aim of this work was to study whether the *apoE* genotype interacts with the quantity and quality of dietary fat, modifying LDL size in young healthy subjects. Healthy subjects ($n = 84$; 66 apoE 3/3, 8 apoE 4/3, 10 apoE 3/2) were subjected to 3 dietary periods, each lasting 4 wk. The first was an SFA-enriched diet (38% fat, 20% SFA), which was followed by a carbohydrate (CHO)-rich diet (30% fat, < 10% SFA, 55% carbohydrate) or a monounsaturated fatty acid (MUFA) olive oil–rich diet (38% fat, 22% MUFA) following a randomized crossover design. At the end of each diet period, LDL particle size and plasma levels of total cholesterol, LDL cholesterol (LDL-C), HDL-C, apoB, apoA-I, and triacylglycerols were determined. LDL particle size was significantly higher ($P < 0.04$) in subjects with the apoE 4/3 genotype compared with those with apoE 3/3 and apoE 3/2 in the basal state. LDL size was smaller ($P < 0.02$) after the CHO diet than after the MUFA or SFA diets. After the CHO diet, a significant increase in LDL particle size ($P < 0.035$) was noted with respect to the MUFA diet in apoE 4/3 subjects, whereas a significant decrease was observed in the apoE 3/3 individuals ($P < 0.043$). In conclusion, a Mediterranean diet, high in MUFA-fat increases LDL particle size compared with a CHO diet, and this effect is dependent of apoE genotypes. J. Nutr. 134: 2517–2522, 2004.

KEY WORDS: • *apoE gene polymorphism* • *diet* • *LDL size*

Apolipoprotein E (apoE)[3] plays an important role in lipid metabolism, both promoting efficient uptake of triglyceride-rich lipoproteins (TRL) from the circulation (1,2) and 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 3 main isoforms (apoE2, apoE3, and apoE4). These proteins determine changes in apoE plasma concentrations and differ in their affinity to its specific receptors (4,5).

The apoE4 variant has been associated with increased LDL production from VLDL, increased uptake of postprandial lipoproteins, increased intestinal absorption of cholesterol, decreased bile acid synthesis, and faster LDL clearance from plasma compared with the apoE3 or apoE2 variants (6–8). The apoE2 allele has been consistently associated with lower LDL cholesterol (LDL-C) and apoB levels and higher triacylglycerol, HDL-C and apoA-I concentrations (9–11).

The highest apoB, total cholesterol (TC) and LDL-C levels associated with the apoE4 isoform (12–15) are related to the intake of diets enriched in saturated fat and cholesterol (16,17). These findings led to an examination of the interaction between lipoprotein responsiveness to dietary manipulation and apoE alleles in a number of studies. However, the results have been controversial (18). Although some studies found a pronounced dietary responsiveness for apoE4 carriers, others reported no difference in response across apoE genotypes to changes in dietary fat or cholesterol content (19–21). Thus, the hyperresponse of LDL-C concentrations associated with the E4 allele occurred only when the fat content in the diet varied (22).

Several studies suggested that the presence of small, dense LDL particles is associated with an increased risk of coronary artery disease (CAD) (23). LDL particle size is dependent on both genetic factors and environmental factors such as dietary fat composition. Low-fat, high-carbohydrate diets decreased mean LDL size compared with high saturated fat diets (24,25); the largest and smallest subfractions decreased in concentration, whereas the intermediate-small fraction increased. Monounsaturated fat diets, slightly reduced (26) or did not affect (27) LDL size compared with saturated fat diets. Overall, it is difficult to provide a clinical interpretation to infer benefit or harm from such changes in LDL sizes during these interventions. On the other hand, several authors indicated that the apoE genotype is a major genetic determinant of LDL size

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[3] Abbreviations used: apo, apolipoprotein; CAD, coronary artery disease, CHO, carbohydrate; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MUFA, monounsaturated fatty acid; TC, total cholesterol; TRL, triacylglycerol rich lipoproteins.

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although results are contradictory. Although some data show that subjects carrying the apoE2 allele have smaller and denser LDL than subjects carrying the apoE4 allele (28), other studies failed to show this relation (29) or even that subjects carrying apoE4 allele have smaller LDL particle diameter than subjects with the apoE2 allele (15,30). Interestingly, even though both apoE phenotype and diet modify LDL size, studies showing the interaction between these 2 factors are scarce or discrepant. Thus, a higher saturated fat intake was associated with smaller LDL particles in apoE2 subjects, and larger LDL particles in apoE4 subjects (31). However, another study showed that when subjects changed from a high- to a low-fat diet, there was a shift from large, buoyant, cholesterol-rich particles, to smaller, denser LDL particles, with progressively greater reductions in levels of larger LDL from apoE3/2 to apoE3/3 to apoE3/4 (32). Therefore, the aim of this work was to examine whether the apoE genotype interacts with the quantity and quality of dietary fat, modifying LDL size in young healthy subjects.

SUBJECTS AND METHODS

Human subjects. A group of healthy young adults (n = 84; 66 apoE 3/3, 8 apoE 4/3, 10 apoE 3/2), including both men (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 from among students at the University of Cordoba. The subjects were 21.55 ± 0.40 y old (mean ± SD). Informed consent was obtained from all participants. All subjects underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrolment. 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 mo before the study. Physical activity and diet, including alcohol consumption, were recorded in a personal log for 1 wk and the data were used to calculate individual energy requirements. The 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-d period during which all subjects consumed a SFA-rich diet, with 15% protein, 47% carbohydrate (CHO) and 38% fat [20% SFA, 12% monounsaturated fatty acid (MUFA) and 6% PUFA]. After this period, volunteers were randomly assigned to 1 of 2 diet sequences. Forty-two subjects consumed a MUFA-rich diet containing 15% protein, 47% CHO and 38% fat (<10% SFA, 6% PUFA, 22% MUFA) for 28 d. This diet was followed for 28 d by consumption of a CHO-rich diet containing 15% protein, 55% carbohydrates and < 30% fat (<10% SFA, 6% PUFA, 12% MUFA). The other 42 subjects consumed the CHO diet before the MUFA diet. The cholesterol content remained constant (<300 mg/d) during the 3 periods. Virgin olive oil comprised 80% of the MUFA diet; it 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 USDA (33) food tables and Spanish food composition tables for local foodstuffs (34). All meals were prepared in the hospital kitchen and were supervised by a dietitian. Lunch and dinner were eaten in the hospital dining room, whereas breakfast and an afternoon snack were eaten in the medical school cafeteria. Menus (n = 14) were prepared with regular solid foods and rotated during the experimental period. Duplicate samples from each menu were collected, homogenized, and stored at −70°C. Protein, fat, and carbohydrate contents of the diet were analyzed by standard methods (35). Dietary compliance was verified by analyzing the fatty acids in LDL-C esters at the end of each dietary period (36). The study took place from January to March to minimize seasonal effects and academic stress.

Lipid analysis, biochemical determinations, and LDL size. Venous blood samples were collected into EDTA-containing (1 g/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 (1500 × g) for 15 min at 4°C within 1 h of venipuncture. To reduce interassay variation, plasma was stored at −80°C and analyzed at the end of the study. Plasma TC and triacylglycerol levels were determined by enzymatic techniques (37,38). HDL-C was determined after precipitation with phosphotungstic acid (39). Apo A-I and apo B were determined by immunoturbidimetry (40). LDL-C concentration was calculated using the Friedewald formula (41). The predominant LDL peak particle diameter (LDL size) was measured from serum samples with polyacrylamide gradient gel electrophoresis as described (42). We identified 2 subclass LDL-phenotypes using the criteria described previously (43): the classical category, phenotype A, is >25.5 nm, and phenotype B is ≤25.5 nm.

DNA amplification and genotyping of apoE. Amplification of a region of 266 bp of the apoE gen was done by PCR with 250 ng of genomic DNA and 0.2 μmol of each oligonucleotide primer (E1, 5′-GAACAACTGACCCCGGTGGCGGAG-3′, and E2, 5′-TCGCGGGCCCCGGCCTGGTACACTGCCA-3′) and 10% dimethyl sulfoxide in 50 μL. DNA was denatured at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 63°C for 1.5 min, and extension at 72°C for 2 min. The PCR product (20 μL) was digested with 10 U of restriction enzyme *CfoI* (BRL) in a total volume of 35 μL. Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

Statistical analysis. ANOVA for repeated measures was used to test for the effects of the apoE gene polymorphism on plasma TC, LDL-C, HDL-C, triacylglycerol, apo A-I, and apo B concentration and LDL size in each dietary stage. Triacylglycerols levels were log transformed before statistical analyses. When statistical significance was found, Tukey's post-hoc comparison test was used to identify group differences. Independent sample t-test was used to detect if women had greater LDL-size and higher HDL-C and apoA-I plasma concentration than men after each dietary period.

The χ^2 test was used to determine whether the replacement of high-fat diets (SFA or MUFA) by a low-fat, high-CHO increased the number of subjects with LDL-phenotype B. Differences were considered significant at $P < 0.05$. Statistical analyses were conducted using the SPSS statistical software, version 8.0 (SPSS).

RESULTS

Significant differences were observed when we compared basal characteristics according to *apoE* genotypes (**Table 1**). Thus LDL particle size and TC, LDL-C, and apoB plasma concentrations were higher in subjects with the apoE 4/3 genotype compared with the apoE 3/3 and apoE 3/2 subjects. The actual composition of the mean daily intake of the participants is shown in **Table 2**. Analysis of LDL-C esters obtained after each dietary period showed good adherence in the different intervention stages. After consumption of the SFA diet, there was a greater ($P < 0.005$) increase in palmitic acid in the LDL-C esters than after the CHO and MUFA diets: 27.3 ± 1.4% compared with 19.8 ± 3.9% and 15.2 ± 0.4%, respectively. There was a greater ($P < 0.05$) increase in oleic acid in the cholesterol esters 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%).

Dietary intervention had significant effects on LDL particle size and plasma TC, LDL-C, HDL-C, apoA-I, and apoB concentrations (**Table 3**), but did not affect the triacylglycerol plasma concentration. In comparison with the SFA diet, the

TABLE 1

Baseline characteristics of plasma lipids and apolipoproteins according to the apoE genotypes[1]

	ApoE 4/3	ApoE 3/3	ApoE 3/2
n	8	66	10
Age, *y*	23.75 ± 2.22	21.45 ± 1.76	21.22 ± 2.44
BMI, *kg/m²*	22.23 ± 2.79	22.69 ± 3.24	23.02 ± 3.89
TC, *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]

[1] Values are mean ± SD. Means in a row with superscripts without a common letter differ, $P < 0.05$.

CHO diet was associated with a decrease in the plasma TC (−0.56 mmol/L, $P < 0.001$), LDL-C (−0.44 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 (−0.07 g/L, $P < 0.002$) concentrations. The MUFA diet had similar effects, with decreases in the concentrations of TC (−0.49 mmol/L, $P < 0.001$), LDL-C (−0.41 mmol/L, $P < 0.001$), HDL-C (−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, compared with the MUFA diet, the CHO diet was associated with a decrease in the plasma concentrations of HDL-C (−0.06 mmol/L, $P < 0.001$) and apoA-I (−0.04 g/L, $P < 0.001$). LDL particle size was lower ($P < 0.02$) after the CHO diet than after the high MUFA diet and high SFA diet.

TABLE 2

Daily intake during each experimental diet period by healthy young adults of differing apoE genotype consuming different quantities and types of fat

Diet	SFA	CHO	MUFA
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

TABLE 3

Plasma lipid levels and LDL-size after dietary intervention in healthy young adults of differing apoE genotype consuming different quantities and types of fat

	SFA	CHO	MUFA
TC, *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[a]

[1] Values are mean ± SD, $n = 84$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

LDL-size was negatively correlated with triacylglycerols levels ($r = -0.234$; $P < 0.0009$). Women had greater ($P < 0.05$) LDL particle size and higher HDL-C and apoA-I plasma concentration than men independently of the quality and quantity of dietary fat (data not shown). The shift from SFA and MUFA diets to the CHO diet increased ($P < 0.0001$) the number of subjects with smaller LDL particle diameters (phenotype B). The increase in number of subjects with phenotype B after the CHO diet was observed in both men and women (**Table 4**).

The *apoE* genotype affected apoB ($P = 0.003$), TC ($P = 0.032$) and LDL-C ($P = 0.023$) concentrations. Thus, apoB, TC and LDL-C plasma concentrations were higher in subjects with the apoE 4/3 genotype, intermediate in apoE 3/3 subjects, and lower in apoE 3/2 subjects after the CHO, SFA, and MUFA diets (**Table 5**). Significant differences ($P < 0.009$) in LDL-C levels were observed between apo4/3 vs apo3/3 and apo3/2 subjects when changing their diet from MUFA to CHO (Table 5). Subjects with apoE 4/3 genotype had an increase of 0.31 ± 0.19 mmol/L after changing their diet from MUFA to CHO, whereas subjects with apoE 3/3 had similar levels of LDL-C and subjects with apoE 3/2 had a decrease of 0.15 mmol/L. LDL-size was larger ($P < 0.05$) in apoE 4/3 subjects than in apoE 3/3 and apoE 3/2 subjects after SFA,

TABLE 4

Number of subjects with differing apoE genotype having the LDL phenotype A and phenotype B after consumption of different quantities and types of fat[1]

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

[1] For a given phenotype, different superscript letters indicate differences between diets, $P < 0.05$.

TABLE 5

Plasma lipids, apoproteins, and LDL-size in young adults 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
	mmol/L			*g/L*		*nm*
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

[1] Values are mean ± SD. Genotype means for a given period without a common letter differ, $P < 0.05$.

CHO, and MUFA diets. In addition, there was a decrease in LDL-size in subjects with apoE 4/3 after changing from a CHO diet to a MUFA diet (0.22 ± 0.15, $P < 0.035$), whereas in subjects with apoE 3/3 there was an increase (0.17 ± 0.06, $P < 0.043$). However, there was no interaction between gender and LDL size by genotype.

DISCUSSION

Our results showed that replacement of a CHO diet by a MUFA diet increased the LDL-size in apoE 3/3 young healthy subjects, whereas it decreased LDL-size in apoE 4/3 subjects.

A diet high in saturated fat contributes to the development of CAD; thus, dietary intervention is recommended to lower plasma lipid levels. However, it is not clear whether saturated fat should be replaced by carbohydrates or monounsaturated fat. In accordance with our results, previous studies indicated that both MUFA and CHO diets reduce TC and LDL-C (44,45). However, when subjects consumed a MUFA diet the levels of HDL-C and apoA-I were higher compared with those consuming a CHO diet. In addition, we observed that low-fat, high-carbohydrate diets compared with high saturated fat decreased the mean LDL size (24,25,31,32). Furthermore, our data confirm the results obtained in a recent study in which no differences in LDL size were observed between MUFA and SFA diets (27). But more importantly, our study shows that a Mediterranean diet enriched in MUFA increases LDL size in comparison with a low-fat, high-carbohydrate diet. Studies employing analysis of LDL subclasses demonstrated heterogeneity of the LDL response to low-fat, high-carbohydrate diets in healthy subjects (46). In individuals with a genetically influenced atherogenic lipoprotein phenotype, characterized by a predominance of small dense LDL (phenotype B), lowering of plasma LDL cholesterol levels by low-fat diets represents a reduction in the numbers of circulating small LDL particles. In contrast, in the majority of healthy individuals with larger LDL (phenotype A), a significant proportion of the low-fat diet-induced reduction in plasma LDL cholesterol is due to depletion of the cholesterol content of LDL particles and the shift from larger to smaller LDL particle diameters. Moreover, with a progressive reduction of dietary fat and isoenergetic substitution of carbohydrate, an increasing number of subjects with phenotype A convert to the phenotype B, as noted here.

One of the most intriguing aspects of the response of plasma cholesterol to diet is how it varies among individuals. The influence of the genetic loci of the principal apolipoproteins such as the AI-CIII-AIV complex and the apoE gene has been studied (22,47,48). In agreement with previous studies (9,10), we observed that the presence of the apoE4 allele is associated with a proatherogenic lipoprotein profile, with increased apoB, TC, and LDL-C plasma concentrations. The effect of the apoE genotypes on plasma lipids is modified by environmental factors such as dietary intake (12,49). Thus, the hyperresponse of LDL-C concentrations associated with the E4 allele occurred only when the fat content in the diet varied (23). We observed that replacement of a CHO diet by a MUFA diet was associated with a significant decrease in LDL-C in apoE 3/4 subjects, whereas the opposite or no effect was observed for apoE 3/2 and apoE 3/3 subjects, respectively.

The relation between apoE genotype and LDL size provided contrasting results (15,26,28–31,50). In accordance with previous studies, we observed that apoE2 subjects had smaller LDL particles than the other groups (29,31,50) and changes in LDL size were inversely correlated with triacylglycerol levels (28). The mechanism by which apoE isoforms might affect LDL particle size is not completely clear. Barballo et al. (50) speculate that this genetic response probably involves the effect of apoE2 on receptor-mediated VLDL clearance. Thus, the decreased uptake of VLDL and VLDL remnants by the B/E receptor in apoE2 subjects leads to higher levels of TRL with an increased conversion into smaller and denser LDL particles. However, other studies reported that apoE2 subjects have similar or even larger LDL particles compared with apoE3 and apoE4 individuals (15,30,31). Environmental factors, such as diet or geographical differences, could explain the variability observed in these studies. In agreement with this hypothesis, some studies examined the effect of apoE gene-diet interaction on LDL-size. Thus, Campos et al. (31) divided free-living Costa Ricans according to their intake of saturated fat and observed that higher saturated fat intake was associated with

smaller LDL particles in apoE2 subjects, and larger LDL particles in apoE4 subjects. However the gene-diet interaction was not significant for LDL particle size. This study was conducted in a population under normal daily conditions without dietary intervention. In our study, subjects were randomly assigned to a dietary intervention study, which means that the results obtained are more reliable. Thus, the replacement of a CHO diet by a MUFA diet increases LDL-size in apoE 3/3, whereas it decreases it in apoE 4/3 subjects. We also observed that LDL-size was larger in apoE 4/3 subjects than in apoE 3/3 and apoE 3/2 subjects after the SFA, CHO, and MUFA diets. However, Dreon et al. (32) found that reduction in dietary fat resulted in a shift from large, buoyant, cholesterol-rich particles to smaller, denser, LDL particles, with progressively greater reductions in levels of larger LDL from apoE 3/2 to apoE 3/3 to apoE 3/4. Their results apply only to reduction in total fat intake, and it is possible that apoE isoforms operate differently in influencing the response to other dietary manipulation, such as substitution of monounsaturated fat or carbohydrates for saturated fat intake.

Studies in vitro demonstrated that oleic acid is a potent stimulator of TRL secretion (51), and test-meal studies found that meals high in oleic acid–rich oils caused a more pronounced, sharper postprandial rise in plasma TRL than SFA-rich meals (52). The apoE4 variant was associated with increased uptake of postprandial lipoproteins compared with the apoE3 or apoE2 variants (6). Therefore, apoE4 subjects would have lower levels of TRL with a decreased conversion into smaller and denser LDL particles (24). A MUFA diet, high in oleic acid from virgin olive oil, could regulate the increased uptake of TRL in apoE4 subjects. This would explain the decrease in LDL particle size observed in our study when apoE4 subjects changed from a CHO diet to a MUFA diet. We observed this effect only in apoE4 subjects likely because carriers of the apoE4 allele have a greater lipid response to dietary changes than individuals not possessing the apoE4 allele (22). Although increased intake of carbohydrates may decrease LDL size (24,25), it is not known whether reduced dietary fat intake contributes to these lipoprotein changes to a lesser extent than the MUFA diet, as we observed. New studies are warranted, therefore, to confirm our results. It is important to note that one of the limitations to genetic association studies is the difficulty in corroborating findings observed in populations with different characteristics. We must be cautious therefore when extrapolating the results to a more general population.

In conclusion, our data indicate that each subject has to be examined and guided individually when dietary recommendations are made. No diet can be recommended unequivocally without knowing more about those being targeted. Even though a MUFA-rich diet increases LDL size compared with a CHO-rich diet, this effect is dependent on apoE genotypes. Thus, the replacement of a CHO diet by a MUFA diet increases LDL-size in apoE 3/3, whereas it decreases it in apoE 4/3 subjects.

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