Environmental Factors Modulate the Effect of the *APOE* Genetic Polymorphism on Plasma Lipid Concentrations: Ecogenetic Studies in a Mediterranean Spanish Population

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To investigate *APOE* gene–environment interaction effects on plasma lipid concentrations, we conducted a cross-sectional study in a Mediterranean Spanish population consisting of 396 men and 513 women aged 18 to 66 years. The frequency of the ε4 variant was 0.071 (95% confidence interval 0.059, 0.082), confirming the lower frequency of this allele in Southern Europe. In general, the carriers of the ε2 variant had lower concentrations ($P < .05$) of total and low-density lipoprotein cholesterol (LDL-C), carriers of the ε3 variant had intermediate concentrations, and carriers of the ε4 variant had higher concentrations ($P < .05$) in both sexes, even after multivariate adjustment for age, body mass index, alcohol consumption, tobacco smoking, physical activity, marital status, and education. However, when the homogeneity of allelic effects according to environmental factors was tested, significant interaction terms were found. In women, an important interaction between alcohol consumption and the *APOE* polymorphism in determining LDL-C concentrations was found ($P < .003$). LDL-C concentrations in female drinkers with the ε2 variant were significantly lower ($P < .014$) than in nondrinkers with the ε2 variant. Likewise, in female drinkers with the ε4 variant, LDL-C concentrations were also significantly ($P < .010$) lower than in nondrinkers with the ε4 variant. Moreover, in female drinkers, LDL-C concentrations did not differ between carriers of the ε4 and the ε3 variants, and in nondrinkers, LDL-C concentrations did not differ between carriers of the ε2 and the ε3 variants. We also found a statistically significant interaction effect ($P < .001$) between the *APOE* polymorphism and physical activity in determining high-density lipoprotein cholesterol concentrations in men. Our results indicate that environmental factors are important modulators of the effect of the *APOE* polymorphism on plasma lipid concentrations.

ATHEROSCLEROSIS is a multifactorial disease in which complex interactions between genetic and environmental factors play an important role.[1-3] Human genome epidemiology aims to elucidate how genes and the environment interact in determining individual susceptibility to multifactorial disease.[4] Genotype–environment interaction refers to the differential effects of the same environment on individuals with different genotypes, or the differential effects of different environments on individuals with the same genotype.[5,6] Variations in candidate genes for lipoprotein metabolism have been associated with lipid disorders and with the development of atherosclerosis. The most comprehensively studied is the apolipoprotein E (apoE) gene locus (*APOE*),[7-11] with its 3 common alleles: ε2, ε3, and ε4. However, few studies have focused on interactions with environmental factors.[12,13] One of the reasons might be that large sample sizes are required for acceptable statistical power to detect such interactions. The average effects attributed to the ε2 allele are decreased low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) concentrations and increased apoE concentrations, whereas the ε4 allele has the opposite effect.[7,8,14,15] However, the associations of these alleles with high-density lipoprotein cholesterol (HDL-C) or triglyceride (TG) concentrations are less clear. In addition, recent reports[16,17] have shown a lack of the effect of *APOE* polymorphism on LDL-C levels, adding support to the set of studies that have suggested that ethnicity and environmental factors modulate the effects of this polymorphism. Moreover, it has been established that the apoE genotype distribution and the related allele frequencies vary among populations around the world.[18,19] In Europe, frequency of the ε4 allele seems to decrease from north to south along with the coronary heart disease (CHD) mortality gradient.[14,20,21] Although the *APOE* genotype distribution has been studied extensively in several European countries, limited information is available for Spain.[22,23] The Spanish genetic pool has been influenced by many populations and immigrations, yielding a high heterogeneity between regions.[24] Paradoxically, the Valencia region, on the Mediterranean coast of Spain, has the highest CHD mortality rate in the country,[25] and there are no data on the *APOE* alleles in this geographic area. Therefore, the aim of this study was to estimate allele frequencies for the *APOE* polymorphism and to investigate the association between this polymorphism and plasma lipid and lipoprotein concentrations, taking into account the interactions with environmental factors in a healthy Mediterranean population from Valencia, Spain.

MATERIALS AND METHODS

Subjects and Study Design

This work is part of a broader population survey on cardiovascular risk factors in the Valencia region, designed to ascertain the prevalence

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of both genetic and environmental CHD risk factors in this population. The Ethics Committee on Human Research of the Valencia University approved the study protocol. In this report, we present data obtained from 909 individuals attending the first cross-sectional examination in 1998 through 1999. Participants were healthy, unrelated subjects residing and working in the region. These subjects were randomly selected from more than 5,000 employees examined in a local medical center. Using a continuously updated computerized population register, a sex-stratified random sample was drawn in 2 groups (50% male, 50% female), with 750 subjects selected from each stratum. Of 1,500 eligible subjects asked to participate, 413 men and 528 women agreed. The participation rate was 55% in men and 70% in women. These figures are unlikely to affect the representativeness of the participants in terms of *APOE* genotype and associations between genotype and lipid traits. Questionnaires were distributed at the time of the medical examination, and participants were invited to complete them. Non-Caucasian individuals (2 men and 1 woman) were excluded from the study at this stage. Of the 938 individuals who completed the questionnaire, 909 (396 men and 513 women) had a DNA sample isolated from blood, and this whole group was used to estimate the prevalence of the *APOE* alleles. In the association analyses with lipid traits, pregnant women (n = 2) and subjects taking any lipid-lowering drugs (18 men and 11 women) were excluded. There were 52 (35 men and 17 women) a posteriori exclusions due to missing data on biochemical or lifestyle variables. Subjects with rare *APOE* variants (6 $\varepsilon 2/\varepsilon 4$ and 1 E2-Christchurch carrier) were also excluded. The final group size in the association analysis was 819 (338 men and 481 women, aged 18 to 66 years).

Sample and Data Collection

Participants were instructed to fast for at least 12 hours before a morning examination. Venous blood was collected during the medical check-up into ehtylenediaminetetraacetic acid (EDTA)–containing glass tubes. Plasma total cholesterol and TG concentrations were determined by use of a Technicon Chem 1 assay (Technicon Instruments, Tarrytown, NY), and HDL-C was measured in the supernatant after precipitation of apoB-containing lipoproteins with heparin-manganese chloride. LDL-C concentrations was calculated according to the equation of Friedewald et al[26] for samples with serum TG concentrations below 400 mg/dL. Coefficients of variation for total cholesterol, HDL-C, and TG were each less than 5%.

Anthropometric measurements were taken using standard techniques: weight with light clothing by digital scales; height without shoes by fixed stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

DNA Extraction and Genotyping

Genomic DNA was isolated from white blood cells by phenol-chloroform extraction and ethanol precipitation. *APOE* genotypes were determined as described by Hixson and Vernier.[27] Quality control for the DNA genotyping was maintained by the use of both positive and negative controls in each set of analyzed samples and by independent assignment of genotypes by 2 laboratory workers and replication of those samples with lack of concordance in the genotyping. The presence of the rare mutation E2-Christchurch[28] in 1 individual was also confirmed by sequence analysis in an ABI Prism Automated DNA Sequencer using the ABI Prism big dye terminator cycle sequencing ready reaction kit.

Questionnaire

The questionnaire concerned gender, ethnicity, nationality, place of residence, date of birth, marital status, type of education, profession, medication, health problems, family history, tobacco use, drinking habits, physical activity, and reproductive history in women. This self-administered questionnaire that had been previously validated in a small sample of this population also contained detailed questions about tobacco smoking. Current smokers were defined as those smoking at least 1 cigarette a day. Former smokers were defined as those who had smoked regularly at least 1 cigarette a day but had not smoked for more than 1 month before the examination. Alcohol consumption was carefully evaluated by a set of 22 questions about the use of alcoholic beverages during workdays and weekends, as previously described.[29] The mean daily ethanol consumption (in grams) was calculated by multiplying the amount consumed (in milliliters) by the percentage of ethanol supplied by each specific beverage. From the reported alcoholic beverages, alcohol consumption was considered as a continuous variable expressed in grams per day. Alcohol consumption was categorized as a drinker variable: nondrinkers (no alcohol consumption) and drinkers (any amount of alcohol consumed). Physical activity was estimated by questions about regular leisure time physical sports (aerobics, basketball, bicycling, gymnastics, running, soccer, squash, swimming, tennis, volleyball, and others), as well as the average number of hours per week spent in each activity. According to the type and time, subjects were categorized as sedentary (no physical exercise), moderate (1 sport less than 3 h/wk) and high (1 sport more than 3 h/wk or more than 2 sports per week). For regression analyses, physical exercise was also dichotomized as sedentary versus active (moderate and high). Marital status was dichotomized as single (living alone and divorced) or living with a partner. Education was classified into 4 categories—primary, secondary, university I (3 years), and university II (5 years or more)—and recoded into 2—university and nonuniversity.

Statistical Analysis

We examined all continuous variables for normality. TG concentrations and alcohol intake were markedly skewed, and these variables were logarithmically and square root transformed, respectively, to improve normality. Allele frequencies were estimated by gene counting, and 95% confidence intervals (CI) were calculated. χ^2 tests were used to test differences between observed and expected frequencies, assuming Hardy-Weinberg equilibrium, and to test differences in percentages between men and women. To assess mean differences between sexes, the Student *t* test was used. For multiple comparisons of means between genotypes, 1-way analysis of variance (ANOVA) with Bonferroni correction was performed. Multivariate linear regression analysis with dummy variables for categorical terms was used to test the null hypotheses of no association between *APOE* polymorphism and lipid and lipoprotein levels. For each lipid and lipoprotein considered, a regression model (model 1) including the *APOE* polymorphism as a categorical variable ($\varepsilon 2$, $\varepsilon 3/\varepsilon 3$, and $\varepsilon 4$ genotypes) was first fitted to data, with the $\varepsilon 3/\varepsilon 3$ genotype as reference. Second, a set of covariates (age and body mass index [BMI]) and factors (smoking, drinking, education, marital status, menopause, physical activity) were included in the multivariate modeling (model 2). Regression coefficients and the proportion of variance attributable to each predictor were estimated from the models. Finally, homogeneity of allelic effects according to environmental factors (smoking, alcohol consumption, physical activity, and education) was tested by introducing the corresponding terms of interaction in the hierarchical linear regression model (model 3). Standard regression diagnostic procedures were used to ensure the appropriateness of these models. To better understand the statistically significant interaction effects, the estimated means of lipid variables were plotted according to environmental and genetic strata. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 9.0; SPSS Inc, Chicago, IL) for Windows.

RESULTS

Descriptive characteristics of all the study subjects who were genotyped for *APOE* (396 men and 513 women) are presented in Table 1. Table 2 shows genotypes and allele frequencies for the observed alleles. In addition to the 3 common alleles, a rare apoE variant (E2-Christchurch [Cys112/Ser136/Arg158]), was detected. For the 3 common alleles, no deviation from Hardy-Weinberg equilibrium was detected in either sex ($\chi^2 = 0.510$, 3 df, $P = .917$ for men; and $\chi^2 = 1.877$, 3 df, $P = .599$ for women). To analyze the influence of *APOE* genotype on lipid and lipoprotein concentrations, subjects were grouped as $\varepsilon 2$ carriers ($\varepsilon 2/\varepsilon 2 + \varepsilon 2/\varepsilon 3$), $\varepsilon 3$ homozygotes ($\varepsilon 3/\varepsilon 3$), and $\varepsilon 4$ carriers ($\varepsilon 3/\varepsilon 4 + \varepsilon 4/\varepsilon 4$). In the association study, individuals taking lipid-lowering drugs, pregnant women, the Christchurch carrier, $\varepsilon 2/\varepsilon 4$ individuals, and subjects with missing data on biochemical or environmental variables were excluded, as indicated in Materials and Methods. Table 3 shows age, BMI, and plasma lipid and lipoprotein levels by *APOE* genotypes and sex in the final group (338 men and 481 women). No significant difference in mean age, HDL-C concentration, or BMI was found between *APOE* genotypes. Mean total cholesterol and LDL-C levels differed significantly ($P < .001$) among the 3 *APOE* genotypes in both men and women; $\varepsilon 2$ carriers had the lowest mean total cholesterol and LDL-C concentrations, and subjects with the $\varepsilon 4$ allele had the highest. An effect of *APOE* genotype was also observed on TG concentrations in both genders. Those with the $\varepsilon 4$ allele had higher mean TG concentrations than those with the $\varepsilon 3/\varepsilon 3$ genotype. However, this effect was much stronger and statistically significant in men than in women. With regard to the $\varepsilon 2$ allele, it did not show any significant effect on TG levels in men or women. To test the influence of menopause on the effect of *APOE* polymorphism, these analyses (results not shown) were carried out stratifying by premenopausal (n = 395) and postmenopausal (n = 86) status. Similar effects on total cholesterol and LDL-C were observed for the $\varepsilon 4$ and $\varepsilon 2$ alleles in both groups; however, in postmenopausal women the $\varepsilon 2$ allele was associated with higher TG concentrations than the $\varepsilon 3$, without reaching statistical significance because of the small number of $\varepsilon 2$ carriers in that subgroup (n = 12). No differences in the effect of the $\varepsilon 4$ allele on TG concentrations were observed between premenopausal and postmenopausal women.

Using linear regression analyses, we calculated the proportion of variance of lipid and lipoprotein levels accounted for by the *APOE* polymorphism to estimate the quantitative effect of alleles $\varepsilon 2$ and $\varepsilon 4$ compared with $\varepsilon 3$ homozygosity. First, the simplest model (model 1) was fitted to estimate the unadjusted regression coefficients for each allele. Second, the independent allelic effect was examined by adding covariates (age, BMI, tobacco smoking, alcohol consumption, physical activity, marital status, and education) to the multivariate regression model (model 2). In men, the *APOE* polymorphism explained 7% ($P < .001$), 0% ($P = .981$), and 3% ($P < .006$) of the variability of plasma LDL-C, HDL-C, and TG concentrations, respectively, in model 1. In women, the explained variance was 5%, 0%, and 1%, respectively. Table 4 shows adjusted regression coefficients for *APOE* alleles and covariates in men and women. Only LDL-C, HDL-C, and TG concentrations were considered as outcome variables. When the set of covariates was included in model 2, the *APOE* polymorphism remained independently associated with LDL-C levels ($P < .01$) in both

Table 1. Demographic, Biochemical, and Lifestyle Characteristics of the Study Subjects From the Mediterranean Spanish Population

	Men (n = 396)	Women (n = 513)	
	Mean (SD)	Mean (SD)	*P**
Age (yr)	37.1 (10.2)	36.3 (10.4)	.227
Body mass index (kg/m^2)	26.3 (3.8)	23.7 (4.0)	<.001
Total cholesterol (mg/dL)	203 (41)	193 (34)	<.001
LDL-C (mg/dL)	134 (33)	121 (30)	<.001
HDL-C (mg/dL)	43 (10)	55 (11)	<.001
TG (mg/dL)	111 (87)	69 (38)	<.001
Current smokers (%)	43.0	43.0	.997
Past smokers (%)	21.7	16.4	.068
Alcohol users (%)	90.4	59.2	<.001
Physical exercise (%)			<.001
Sedentary	50.7	60.5	
Moderate	27.3	28.3	
High	22.0	11.2	
Daily walkers (%)	72.0	72.6	.867
Education (%)			.012
Primary	33.2	27.8	
Secondary	27.8	26.5	
University I (3 yr)	17.9	33.7	
University II (25 yr)	21.7	12.0	
Marital status (%)			.008
Married	62.4	53.4	
Single	37.6	46.6	
Premenopausal (%)	—	82.0	
Oral contraceptive users (%)	—	15.9	
Taking lipid lowering drugs (%)	4.9	2.2	.030
Individuals with any missing data (%)	8.8	3.3	.011

* Comparison between men and women; Student *t* test for comparison of means, χ^2 test for percentages.

Table 2. Genotype Distribution and Allele Frequency of *APOE* Polymorphism by Sex in the Mediterranean Spanish Population

	Total (n = 909)	Men (n = 396)	Women (n = 513)
Genotypes, n (%)			
$\varepsilon 2/\varepsilon 2$	4 (0.4)	2 (0.5)	2 (0.4)
$\varepsilon 2/\varepsilon 3$	90 (9.9)	39 (9.8)	51 (9.9)
$\varepsilon 3/\varepsilon 3$	687 (75.6)	294 (74.2)	393 (76.6)
$\varepsilon 3/\varepsilon 4$	115 (12.7)	52 (13.1)	63 (12.3)
$\varepsilon 4/\varepsilon 4$	4 (0.4)	3 (0.8)	1 (0.2)
$\varepsilon 2/\varepsilon 4$	6 (0.7)	4 (1.0)	2 (0.4)
$\varepsilon 3$/E2-Christchurch	1 (0.1)	1 (0.3)	0 (0.0)
Allele frequency			
$\varepsilon 2$ Allele	0.057	0.059	0.055
95% (CI)	(0.046-0.067)	(0.043-0.075)	(0.041-0.068)
$\varepsilon 3$ Allele	0.871	0.862	0.878
95% (CI)	(0.855-0.886)	(0.838-0.886)	(0.858-0.898)
$\varepsilon 4$ Allele	0.071	0.078	0.066
95% (CI)	(0.059-0.082)	(0.059-0.097)	(0.051-0.081)

NOTE. Differences by sex across *APOE* genotypes were nonsignificant. $P = .612$ (χ^2 test).

Table 3. Plasma Lipid and Lipoprotein Levels by *APOE* Genotypes and Sex in the Mediterranean Spanish Population

	ε2 (n = 40 men, 49 women)	ε3 (n = 250 men, 373 women)	ε4 (n = 48 men, 59 women)		
	Mean (SD)	Mean (SD)	Mean (SD)	*P**	*P* Trend
Age (yr)					
Men	37.4 (12.9)	36.8 (10.0)	38.4 (10.2)	.579	.648
Women	36.7 (10.3)	36.0 (10.3)	36.1 (10.2)	.878	.728
Total cholesterol (mg/dL)					
Men	177 (31)‡,§	202 (38)†,§	220 (36)†,‡	<.001	<.001
Women	176 (35)‡,§	191 (32)†,§	209 (37)†,‡	<.001	<.001
LDL-C (mg/dL)					
Men	110 (27)‡,§	134 (32)†	144 (32)†	<.001	<.001
Women	105 (31)‡,§	120 (28)†,§	133 (34)†,‡	<.001	<.001
HDL-C (mg/dL)					
Men	43 (12)	43 (9)	43 (13)	.982	.909
Women	56 (10)	55 (11)	55 (11)	.842	.648
TG (mg/dL)					
Men	109 (66)	107 (85)§	142 (115)‡	.006	.031
Women	69 (37)	68 (34)	77 (45)	.152	.189
BMI (kg/m^2)					
Men	26.0 (3.8)	26.2 (3.8)	26.5 (3.9)	.800	.510
Women	23.5 (3.5)	23.5 (4.3)	23.0 (3.7)	.649	.661

* *P* value obtained by ANOVA for the global comparison between genotypes.

† $P < .05$ compared with the ε2 group, Bonferroni post hoc test.

‡ $P < .05$ compared with the ε3 group, Bonferroni post hoc test.

§ $P < .05$ compared with the ε4 group, Bonferroni post hoc test.

sexes. Compared with model 1, the effect of the ε2 allele was practically unchanged; however, in men, the association of the ε4 allele was attenuated ($P = .054$). In women, the effects of the ε2 and ε4 alleles were highly independent of the adjustment for covariates.

Next, we tested the possible interaction effect between the *APOE* polymorphism and environmental factors separately for each lipid outcome, entering in each multivariate model (model 2) the corresponding interaction term (model 3). The interaction terms were added to the models, including the main effects and all the confounders. Table 5 shows *P* values for the interaction effects between *APOE* polymorphism and lifestyle variables (alcohol consumption, tobacco smoking, physical activity, and education) in men and women. These factors were examined as dichotomous to increase the statistical power. In men, no statistically significant interaction effects were detected between *APOE* genotype and lifestyle variables for LDL-C or TGs. Highly statistically significant interaction effects between the *APOE* polymorphism and alcohol consumption ($P < .002$) and physical activity ($P < .001$) were obtained for HDL-C levels in men. In women, the most prominent interaction effect was ob-

Table 4. Association of *APOE* Polymorphism With Lipid and Lipoprotein Levels

	LDL-C				HDL-C				TG			
	Men		Women		Men		Women		Men		Women	
	B* (SE)	*P*	B* (SE)	*P*	B* (SE)	*P*	B* (SE)	*P*	B* (SE)	*P*	B* (SE)	*P*
APOE genotype		<.002		<.001		.851		.752		.056		.021
ε3 (reference)	—		—		—		—		—		—	
ε2	−21.6 (5.2)	<.001	−16.5 (4.3)	<.001	−0.8 (1.7)	.640	0.8 (1.7)	.651	9.2 (17.2)	.339	−0.3 (5.9)	.901
ε4	9.2 (4.8)	.054	14.1 (3.9)	<.001	−0.1 (1.5)	.953	−0.8 (1.5)	.608	30.8 (15.8)	.020	16.2 (5.4)	.006
BMI (kg/m^2)	0.2 (0.5)	.745	0.9 (0.3)	.002	−0.8 (0.2)	<.001	−0.8 (0.1)	<.001	6.5 (1.6)	<.001	2.7 (0.5)	<.001
Nonsmoking *v* smoking	−9.0 (3.5)	.010	−1.9 (2.6)	.479	3.6 (1.2)	.001	2.9 (1.0)	.006	−24.2 (11.6)	.008	−3.1 (3.6)	.259
Nondrinking *v* drinking	−4.2 (6.0)	.480	2.1 (2.7)	.445	−5.4 (1.9)	.004	0.2 (1.1)	.824	0.6 (19.8)	.469	−1.5 (3.8)	.467
Sedentary *v* active	0.2 (3.5)	.944	0.4 (2.6)	.889	−0.3 (1.1)	.792	0.1 (1.0)	.900	24.1 (11.8)	.075	11.5 (3.7)	.002
Single *v* married	−5.6 (4.2)	.183	2.4 (2.8)	.382	1.4 (1.4)	.297	2.6 (1.1)	.017	−9.8 (14.2)	.624	0.5 (3.9)	.989
R^2 of the model†	0.21	<.001	0.24	<.001	0.16	<.001	0.11	<.001	0.17	<.001	0.18	<.001

NOTE. Adjusted regression coefficients are presented for 338 men and 481 women from the Mediterranean Spanish population using multiple linear regression models.

* B: Regression coefficient (in mg/dL).

† Regression models (model 2) included the following variables: *APOE* genotype, BMI, tobacco smoking, alcohol consumption, physical activity, marital status, age, and education.

Table 5. *P* Values for the Interaction Effects Between *APOE* Genotype and Lifestyle Factors on Lipid and Lipoprotein Levels in the Mediterranean Spanish Population

	Men (n = 338)			Women (n = 481)		
Model 2 + Interaction Terms†	LDL-C	HDL-C	TG	LDL-C	HDL-C	TG
APOE genotype + alcohol consumption	0.330	0.002	0.906	0.003	0.236	0.878
APOE genotype + tobacco smoking	0.354	0.353	0.145	0.619	0.289	0.293
APOE genotype + physical activity	0.704	0.001	0.380	0.882	0.944	0.934
APOE genotype + education	0.708	0.336	0.902	0.218	0.404	0.404

NOTE. Multiple hierarchial linear regression models in men and women.

* Interaction term between the 2 indicated variables.

† The statistical significance of interaction terms in model 3 (model 2 + interaction terms) was tested by including the indicated interaction term in model 2 for each lipid or lipoprotein variable. Lifestyle factors were considered as dichotomous variables, as indicated in Materials and Methods.

tained with alcohol consumption ($P < .003$) in the analysis of LDL-C.

Because these gene–environment interaction effects are considered effect modifiers, to estimate the direction of the effect, regression coefficients for each level of the interaction terms were examined in the multivariate regression model (results not shown). Estimated means for each lipid level were computed from the adjusted models. Adjusted mean values were compared across the different genotypes or environmental factors as indicated. Figure 1A shows the modification of the effect of *APOE* genotypes on LDL-C concentrations by alcohol consumption in women. After adjustment for covariates, LDL-C levels in 2 female drinkers were lower than in 2 nondrinkers (96 ± 27 and 117 ± 32 mg/dL, respectively; $P < .014$). Moreover, in ε4 female drinkers, LDL-C concentrations were also lower than in ε4 nondrinkers (128 ± 30 and 148 ± 31 mg/dL, respectively; $P < .010$). In addition, when LDL-C levels across *APOE* genotypes were examined in relation to drinking status, LDL-C did not differ between the ε3 and ε4 groups (121 ± 27 and 127 ± 30 mg/dL, respectively; $P = .151$) in drinkers. In nondrinkers, LDL-C concentrations did not differ between the ε2 and ε3 groups (117 ± 32 and 122 ± 30 mg/dL, respectively; $P = .468$). When we examined a possible dose response in the effect of alcohol consumption in women considering the 3 categories of drinking status (nondrinkers, low consumption, and moderate-high consumption, based on mean of alcohol intake in drinkers of 5 g/d) in a multiple linear regression model, a clear trend was found, with LDL-C concentrations decreasing with higher concentrations of alcohol intake (Fig 1B).

In men, the most significant gene–environment interaction effect was found between physical activity and the *APOE* polymorphism on HDL-C concentrations ($P < .001$). Figure 2 shows the modification of the effect of this polymorphism on HDL-C concentrations in men according to their physical activity (sedentary *v* active). HDL-C levels differed across *APOE* genotypes in sedentary men, with the ε2 carriers showing higher levels than ε4 carriers (P for trend $< .012$). Conversely, in active men, we observed the opposite effect ($P < .019$), with ε4 carriers showing the highest HDL-C concentrations. The greatest effect of physical activity was noted among ε4 subjects, with mean HDL-C values of 38 ± 8 and 48 ± 15 mg/dL ($P < .006$) for sedentary and active subjects, respectively.

Finally, taking into account that the regression models that have been constructed may have limited power to detect some interactions, men and women were combined to increase the power. All regression models presented in Table 5 were fitted for men and women together including a sex variable. In these multiple hierarchical regression models, we first tested the interaction term among gender, *APOE* genotype, and environmental factor. If this interaction term was statistically significant, men and women cannot be combined. We found a statistically significant gender–gene–environment interaction only in the case of physical activity ($P < .021$) in determining HDL-C concentrations. The interaction term among gender, *APOE* genotype, and alcohol consumption in determining LDL-C levels was not significant ($P = .178$). The statistical significance for the interaction term between alcohol consumption (drinkers and nondrinkers) and *APOE* genotype in determining LDL-C levels by combining men and women was $P < .010$. These results indicated that in men, alcohol drinking also modified the effect of the *APOE* genotype on LDL-C levels; however, because of the small prevalence of nondrinkers in our population, we cannot detect such interaction.

No additional statistically significant interaction terms between *APOE* genotype and environmental variables were detected in the other models combining men and women.

DISCUSSION

The *APOE* gene has been proposed as a model candidate for study of gene–environment interaction effects.[2,30] Consistent with this proposal, we found in our population that several lifestyle factors modify the effect of the common *APOE* alleles on lipid traits.

Some studies have shown the heterogeneity of *APOE* allele frequencies around the world.[15,18,31,32] This variation is of particular interest in Europe because it parallels the CHD mortality pattern.[20] The frequency of the ε4 allele follows a clear north to south gradient, ranging from more than 0.20 in Sweden and Finland to less than 0.07 in Greece and Italy.[14,20,33] Spain, as a southern European country, should have a low prevalence of the ε4 allele, but studies analyzing this topic are scarce and have had small sample sizes. Our results, obtained in a large and randomly selected sample from the Mediterranean Spanish population, confirms the under-representation of this allele in Southern Europe. Although the ε4 allele frequency estimated in the present study is the lowest reported in Spain,

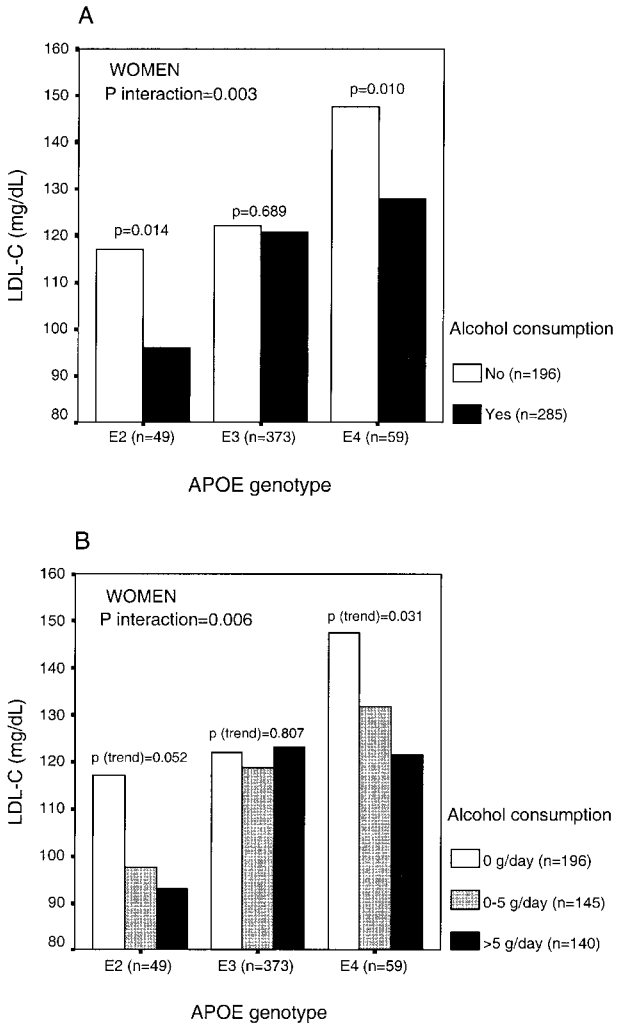

Fig 1. Interaction between *APOE* polymorphism and alcohol consumption in predicting LDL-C levels in women, considering (A) a dichotomous variable or (B) a variable with 3 categories. Estimated means for LDL-C by *APOE* genotype and alcohol consumption were adjusted for age, BMI, tobacco smoking, physical activity, marital status, and education, as indicated in Materials and Methods. The indicated *P* values for interaction corresponded to statistical significance for the interaction terms between *APOE* genotype and the alcohol consumption variable in the multivariate regression models (model 3). *P* values shown for each genotype correspond to the comparison between the categories of alcohol consumption (28 drinkers and 21 nondrinker in the ε2 group; 218 drinkers and 155 nondrinkers in the ε3 group; and 39 drinkers and 20 nondrinkers in the ε4 group).

the differences from other Spanish regions were not statistically significant,[23,34] suggesting that this allele may be homogeneously low in the country. The frequency of the ε2 allele in this study (0.057) differed from that reported in Madrid[34] and was one of the lowest in Europe, along with those of Greeks (0.054) and the Sardinian populations (0.050).[35] However, when comparing allele frequencies, we studied a working population aged 18 to 66 years that may not be representative of the total population.

Despite the significant geographic differences in allele frequencies, most studies have found that the ε4 allele is associated with increased levels of total cholesterol and LDL-C compared with ε3, and the opposite is true for ε2.[7-9,14,15,36,37] In our study, which included young healthy subjects to minimize the effects of medication and aging on serum lipid profiles, the ε2 carriers have lower total cholesterol concentrations, ε3/ε3 subjects have intermediate concentrations, and ε4 carriers have a higher concentrations in both sexes, even after adjustment for covariates. We found a similar association for LDL-C in women; however, in men, the difference between ε4 and ε3/ε3 carriers was smaller and borderline significant. Some previous studies have indicated sex differences in the effect of *APOE* polymorphism on plasma lipid and lipoprotein levels.[34,36-39] In the Framingham Study, women, especially postmenopausal women, had greater effects of *APOE* polymorphism on LDL-C than did men.[37] In the other Spanish study in healthy subjects from Madrid,[34] no effect of the *APOE* polymorphism on LDL-C concentrations was detected in men. Some authors have suggested that a hormonal influence is responsible for the different effects observed in women and men. Alternatively, there may be additional lifestyle factors, differentially distributed in men and women, that modulate the effect of *APOE* polymorphism on LDL-C levels. These environmental factors may also be responsible for the differences in the effect of the *APOE* polymorphism on lipid traits reported across several populations around the world. Among environmental factors, diet has been proposed as a plausible modulator of allele effects, and several authors have argued a gene–diet interaction to explain the lack of effect of the ε4 allele to increase LDL-C concentrations as observed by Deiana et al[17] in the island

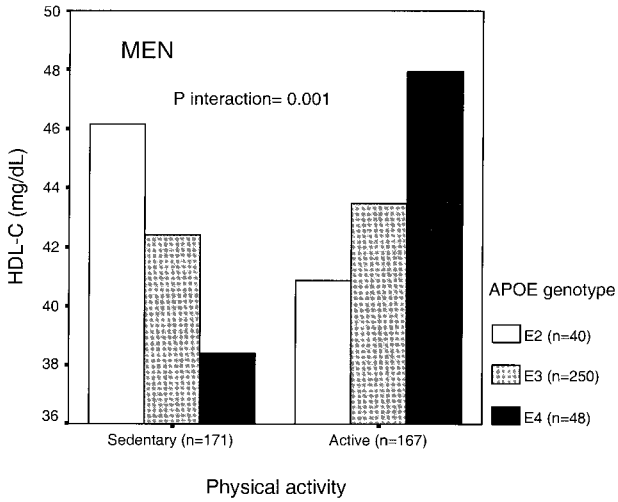

Fig 2. Interaction between *APOE* polymorphism and physical activity in predicting HDL-C levels in men (18 sedentary and 22 active in the ε2 group; 128 sedentary and active in the ε3 group; 25 sedentary and 23 active in the ε4 group). Estimated means for HDL-C by *APOE* genotype and physical activity were adjusted for age, BMI, tobacco smoking, alcohol consumption, marital status, and education as indicated in Materials and Methods. The indicated *P* value for interaction corresponds to the statistical significance for the interaction term between the *APOE* genotype and physical activity in the corresponding multivariate regression model (model 3).

population of Sardinia, by Kamboh et al[40] in the Evenki Herders of Siberia, or by Aguilar et al[16] in a Native American rural population in western Mexico. Other environmental factors such as tobacco smoking, alcohol consumption, physical activity, and education also could interact with the allele effects affecting lipid traits. However, few studies have focused on the interactions between apoE polymorphism and this wide spectrum of environmental factors. One of the reasons might be that large sample sizes are required for acceptable power in detection of such interaction effects. The power of the test to detect interactions depends on the number of observations, the allele frequencies, the proportion of subjects in both genetic and environmental conditions, and the type of interaction. Taking into account the sample size calculations according to the type of interaction on continuous traits proposed by van den Oord,[41] our sample size was large enough to detect type 1a, type 5, and type 6 interactions by sex in the case of dichotomous environmental variables, except for alcohol consumption in men, because of the small prevalence of nondrinkers. Larger sample sizes are needed for detection of other interaction types.

Our results show that of the all lifestyle factors analyzed in women, only the interaction of alcohol consumption with the *APOE* polymorphism resulted in a statistically significant effect in the determination of LDL-C levels. Assuming that the usual effect of the $\varepsilon 2$ allele is to decrease LDL-C concentrations, female nondrinkers carrying the $\varepsilon 2$ allele had increased LDL-C levels that reached mean values similar than those detected in $\varepsilon 3/\varepsilon 3$ women. Nondrinker status in $\varepsilon 4$ carriers was also associated with higher LDL-C concentrations than in $\varepsilon 4$ drinkers. The mechanism by which alcohol consumption may modify the effect of *APOE* genotype on LDL-C levels is not known, but it may be related to changes in absorption of dietary cholesterol and/or down-regulation of the hepatic LDL receptors.[7] On the other hand, these observations may be related to the beneficial effect of moderate alcohol consumption on cardiovascular risk.[42,43] This is the first time that the interaction between the *APOE* genotype and alcohol consumption has been demonstrated in women. In men, 1 previous study has reported an interaction between the *APOE* polymorphism and alcohol consumption in determining blood pressure levels.[44] Recently, in the Framingham Offspring Study we reported an interaction between the *APOE* polymorphism and alcohol consumption on LDL-C concentrations in men.[45] In the present study, although the interaction effects between this genetic polymorphism and alcohol consumption were not statistically significant in men, when men and women were combined in the multivariate regression model, the interaction term reached statistical significance, indicating a similar effect of this interaction in both sexes. However, the small prevalence of nondrinking men in the Mediterranean Spanish population largely affects the statistical power of this test in men.

Although in the present study no association was found between *APOE* polymorphism and HDL-C concentrations when all subjects were analyzed, some statistically significant interactions between this polymorphism and environmental factors were observed. The nonassociation of *APOE* alleles with HDL-C levels has been consistent among studies in healthy populations.[17,23,32,45-47] However, a meta-analysis[48] showed that HDL-C concentration was influenced by the *APOE* genotype. Recently, some studies in healthy populations[14,15,36,49] have observed that the $\varepsilon 2$ allele was associated with increased mean HDL-C concentrations, whereas the opposite was observed for the $\varepsilon 4$ allele. However, important sex differences have been reported for this association. Robitaille et al[36] found no relationship between the *APOE* polymorphism and HDL-C levels in women, whereas in the same population a highly significant association was observed in men. In contrast, in the CARDIA study,[15] the association was higher in women. These results are in agreement with the interactions between some environmental factors (physical activity and alcohol consumption) and the *APOE* genotype in determining HDL-C concentrations found in this study. Our study showed a statistically significant interaction between *APOE* polymorphism and physical activity on HDL-C concentrations in men. When 2 groups where considered, HDL-C concentrations in active men were higher in $\varepsilon 4$ carriers. The opposite was observed for sedentary men. In several experimental studies, it has been suggested that *APOE* genotypes might modify the relationship of physical activity to lipid levels[50,51] but there are no large observational studies analyzing this association or possible additional differences by sex.

Regarding the association between *APOE* polymorphism and TG concentrations, statistically nonsignificant interaction effects with environmental factors were found in the present study. Because the standard deviation for TG levels was higher than for the other lipid variables, a lack of statistical power to detect such interactions could not be discarded. Presently, the association of *APOE* genotype with TG concentrations is still controversial and depends greatly on the demographic characteristics of the population.[14,17,23,36] Based on the results of a meta-analysis, Dallongeville et al[48] showed that TG levels were higher in subjects carrying the $\varepsilon 2$ or $\varepsilon 4$ allele. Our results showed a clear effect of the $\varepsilon 4$ allele, but the increasing effect of $\varepsilon 2$ was not detected. Such conflicting results strongly suggest that the link between *APOE* polymorphism and TG concentrations also involves many interactions with environmental factors.

In summary, the findings of the present study, which was performed in a healthy Mediterranean population, showed that environmental factors modulate the effect of the *APOE* polymorphism on lipid concentrations, explaining the heterogeneity of the magnitude of the allelic effect estimated among different populations.

REFERENCES

1. Hegele RA: Gene-environment interactions in atherosclerosis. Mol Cell Biochem 113:177-186, 1992

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

3. Tall A, Welch C, Applebaum-Bowden D, et al: Interaction of diet and genes in atherogenesis. Report of an NHLBI working group. Arterioscler Thromb Vasc Biol 17:3326-3331, 1997

4. Khoury MJ: Genetic epidemiology and the future of disease prevention and public health. Epidemiol Rev 19:175-180, 1997

5. Andrieu N, Goldstein AM: Epidemiologic and genetic ap-

proaches in the study of gene-environment interaction: an overview of available methods. Epidemiol Rev 20:137-147, 1998

6. Ellsworth DL, Hallman DM, Boerwinkle E: Impact of the Human Genome Project on epidemiologic research. Epidemiol Rev 19:3-13, 1997

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

8. Wilson PWF, Myers RH, Larson MG, et al: Apolipoprotein E alleles, dyslipidemia, and coronary heart disease: The Framingham Offspring study. JAMA 272:1666-1671, 1994

9. Wilson PWF, Schaefer EJ, Larson MG, et al: Apolipoprotein E alleles and risk of coronary disease—A meta-analysis. Arterioscler Thromb Vasc Biol 16:1250-1255, 1996

10. Cumming AM, Robertson FW: Polymorphism at the apoprotein-E locus in relation to risk of coronary disease. Clin Genet 25:310-313, 1984

11. Davignon J, Cohn JS, Mabile L, et al: Apolipoprotein E and atherosclerosis: Insight from animal and human studies. Clin Chim Acta 286:115-143, 1999

12. Zerba KE, Ferrell RE, Sing CF: Genotype-environment interaction: Apolipoprotein E (*ApoE*) gene effects and age as an index of time and spatial context in the human. Genetics 143:463-478, 1996

13. Boer JMA, Ehnholm C, Menzel H-J, et al: Interaction between lifestyle-related factors and the apoE polymorphism on plasma lipids and apolipoproteins. The EARS study. Arterioscler Thromb Vasc Biol 17:1675-1681, 1997

14. Tiret L, de Knijff P, Menzel H-J, et al: ApoE polymorphism and predisposition to coronary heart disease in youths of different European populations: The EARS study. Arterioscler Thromb 14:1617-1624, 1994

15. Howard BV, Gidding SS, Liu K: Association of apolipoprotein E phenotype with plasma lipoproteins in African-American and white young adults. Am J Epidemiol 148:859-868, 1998

16. Aguilar CA, Talavera G, Ordovas JM, et al: The apolipoprotein E4 allele is not associated with an abnormal lipid profile in a Native American population following its traditional lifestyle. Atherosclerosis 142:409-414, 1999

17. Deiana L, Pes GM, Carru C, et al: Lack of influence od apolipoprotein E4 on lipoprotein levels in the island population of Sardinia. Eur J Clin Invest 28:290-294, 1998

18. Hallman DM, Boerwinkle E, Saha N, et al: The apolipoprotein E polymorphism: A comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49:338-349, 1991

19. Gerdes LU, Klausen IC, Sihm I, et al: Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the World. Genet Epidemiol 9:155-167, 1992

20. Lucotte G, Loirat F, Hazout S: Pattern of gradient of apolipoprotein E allele *4 frequencies in western Europe. Hum Biol 69:253-262, 1997

21. Sans S, Kesteloot H, Kromhout D: The burden of cardiovascular diseases mortality in Europe. Task Force of the European Society of Cardiology on cardiovascular mortality and morbility statistics in Europe. Eur Heart J 18:1248, 1997

22. Gené M, Moreno P, Esquerra M, et al: Low apolipoprotein E E4 allele frequency in the population of Catalonia (Spain) determined by PCR-RFLP and laser fluorescent sequencer. Eur J Epidemiol 13:841-843, 1997

23. Muros M, Rodríguez-Ferrer C: Apolipoprotein E polymorphism influence on lipids, apolipoproteins and Lp(a) in a Spanish population underexpressing apo E4. Atherosclerosis 121:13-21, 1996

24. Arnaiz-Villena A, Martinez-Laso J, Alonso-García J: Iberia: Population genetics, anthropology, and linguistics. Hum Biol 71:725-743, 1999

25. Rodriguez-Artalejo F, Banegas JR, García Colmenero C, et al: Lower consumption of wine and fish as a possible explanation for higher ischaemic heart disease mortality in Spain's Mediterranean region. Int J Epidemiol 25:1196-1201, 1996

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

27. Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31:545-548, 1990

28. Pocovi M, Cenarro A, Civeira F, et al: Incomplete dominance of type III Hyperlipoproteinemia is associated with the rare apolipoprotein E2 (Arg136→Ser) variant in multigenerational pedigree studies. Atherosclerosis 122:33-46, 1996

29. Corella D, Sáiz C, Guillén M, et al: Association of TaqIB polymorphism in the cholesteryl ester transfer protein gene with plasma lipid levels in a healthy Spanish population. Atherosclerosis 152:367-376, 200

30. Ellsworth DL, Sholinsky P, Jaquish C, et al: Coronary heart disease. At the interface of molecular genetics and preventive medicine. Am J Prev Med 16:122-133, 1999

31. Kamboh MI, Bhatia KK, Ferrel RE: Genetic studies of human apolipoproteins XII. Population genetics of apolipoproteins in Papua New Guinea. Am J Hum Biol 2:17-23, 1990

32. Zaman MM, Ikemoto S, Yoshiike N, et al: Association of apolipoprotein genetic polymorphism with plasma cholesterol in a Japanese rural population. The Shibata study. Arterioscler Thromb Vasc Biol 17:3495-3504, 1997

33. Cariolou MA, Kokkofitou A, Manoli P, et al: Underexpression of the apolipoprotein E2 and E4 alleles in the Greek Cypriot population of Cyprus. Genet Epidemiol 12:489-497, 1995

34. Gómez-Coronado D, Alvarez JJ, Entrala A, et al: Apolipoprotein E in men and women from a Spanish population: Allele frequencies and influence on plasma lipids and apolipoproteins. Atherosclerosis 147:167-176, 1999

35. Corbo RM, Scacchi R, Mureddu L, et al: Apolipoprotein B, apolipoprotein E, and angiotensin-converting enzyme polymorphism in 2 Italian populations at different risk for coronary artery disease and comparison of allele frequencies among European populations. Hum Biol 71:933-945, 1999

36. Robitaille N, Cormier G, Couture C, et al: Apolipoprotein E polymorphism in a French Canadian Population of Northeastern Quebec: Allele frequencies and effects on blood lipid and lipoprotein levels. Hum Biol 68:357-370, 1996

37. Schaefer EJ, Lamon-Fava S, Johnson S, et al: Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels: Results from the Framingham Offspring study. Arterioscler Thromb 14:1105-1113, 1994

38. Kamboh MI, Aston CE, Hamman RF: The relationship of APOE polymorphism and cholesterol levels in normoglycemic and diabetic subjects in a biethnic population from the San Luis Valley, Colorado. Atherosclerosis 112:145-159, 1995

39. Mahley RW, Palaoglu KE, Atak Z, et al: Turkish heart study: Lipids, lipoproteins, and apolipoproteins. J Lipid Res 36:839-859, 1995

40. Kamboh MI, Crawford MH, Aston CE, et al: Population distribution of APOE, APOH, and APOA4 polymorphisms and their relationships with quantitative plasma lipid levels among the Evenki Herders of Siberia. Hum Biol 68:231-243, 1996

41. van den Oord EJ: Method to detect genotype-environment interactions for quantitative trait loci in association studies. Am J Epidemiol 150:1179-1187, 1999

42. Kiechl S, Willeit J, Rungger G, et al: Alcohol consumption and atherosclerosis: What is the relation? Prospective results from the Bruneck study. Stroke 29:900-907, 1998

43. McElduff P, Dobson AJ: How much alcohol and how often?

population based case-control study of alcohol consumption and risk of major coronary event. Br Med J 314:1159-1164, 1997

44. Kauma H, Savolainen MJ, Rantala A, et al: Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in middle-aged men. Am J Hypertens 11:1334-1343, 1998

45. Corella D, Tucker K, Lahoz C, et al: Alcohol drinking determines the effect of the APOE locus on low-density lipoprotein cholesterol concentrations in male subjects: The Framingham Offspring study. Am J Clin Nutr 73:736-745, 2001

46. Kamboh MI, Evans RW, Aston CE: Genetic effect of apolipoprotein(a) and apolipoprotein E polymorphisms on plasma quantitative risk factors for coronary heart disease in American black women. Atherosclerosis 117:73-81, 1995

47. Pablos-Méndez A, Mayeux R, Ngai C, et al: Association of Apo E polymorphism with plasma lipid levels in a multiethnic elderly population. Arterioscler Thromb Vasc Biol 17:3534-3541, 1997

48. Dallongeville J, Lussier-Cacan S, Davignon J: Modulation of plasma triglyceride levels by apoE phenotype: A meta-analysis. J Lipid Res 33:447-454, 1992

49. Braeckman L, De Bacquer D, Rosseneu M, et al: Apolipoprotein E polymorphism in middle-aged Belgian men: Phenotype distribution and relation to serum lipids and lipoproteins. Atherosclerosis 120:67-73, 1996

50. Taimela S, Lehtimäki T, Porkka KVK, et al: The effect of physical activity on serum total and low-density lipoprotein cholesterol concentrations varies with apolipoprotein E phenotype in male children and young adults: The cardiovascular risk in young Finns study. Metabolism 45:797-803, 1996

51. Hagberg JM, Ferrel RE, Katzel LI, et al: Apolipoprotein E genotype and exercise training-induced increases in plasma high-density lipoprotein (HDL) and HDL_2-cholesterol levels in overweight men. Metabolism 48:943-945, 1999