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ScienceDirect
Journal of Nutritional Biochemistry 22 (2011) 487–494
Journal of Nutritional Biochemistry

Saturated fat intake and alcohol consumption modulate the association between the *APOE* polymorphism and risk of future coronary heart disease: a nested case-control study in the Spanish EPIC cohort☆,☆☆

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Received 30 December 2009; accepted 1 April 2010

Abstract

The association is still not clear between the common *APOE* polymorphism and coronary heart disease (CHD) risk, nor its modulation by diet. Thus, our aim was to study the association between the *APOE* genotypes and incident CHD and how dietary fat and alcohol consumption modify these effects. We performed a nested case-control study in the Spanish European Prospective Investigation into Cancer and Nutrition cohort. Healthy men and women (41 440, 30–69 years) were followed up over a 10-year period, with the incident CHD cases being identified. We analyzed 534 incident CHD cases and 1123 controls. *APOE*, dietary intake and plasma lipids were determined at baseline. The *APOE* polymorphism was significantly associated with low-density lipoprotein cholesterol (LDL-C), and gene–alcohol interactions in determining LDL-C were detected. In the whole population, the E2 allele was significantly associated with a lower CHD risk than E3/E3 subjects [odds ratio (OR), 0.58; 95% confidence interval (CI), 0.38–0.89]. The E4 allele did not reach statistical significance vs. E3/E3 (OR, 1.17; 95% CI, 0.88–1.58). However, saturated fat intake modified the effect of the APOE polymorphism in determining CHD risk. When saturated fat intake was low (<10% of energy), no statistically significant association between the *APOE* polymorphism and CHD risk was observed (P=.682). However, with higher intake (≥10%), the polymorphism was significant (P=.005), and the differences between E2 and E4 carriers were magnified (OR for E4 vs. E2, 3.33; 95% CI, 1.61–6.90). Alcohol consumption also modified the effect of the *APOE* on CHD risk.

In conclusion, in this Mediterranean population, the E2 allele is associated with lower CHD risk, and this association is modulated by saturated fat and alcohol consumption.

Keywords: Nutrigenetics; *APOE*; Saturated fat; Alcohol; LDL-C

☆ This study was funded by a research grant (FIS) of the Spanish Ministry of Health (PI041224; PI042342; PI041822; PI042188); RETIC (RD06/0020 and RD07/0067/0006); CIBER OBN 06/03; CIBERESP and the Spanish Regional Governments of Andalusia, Asturias, Basque Country, Murcia and Navarra and the Catalan Institute of Oncology.

☆☆ None of the funding sources played a role in the design, collection, analysis or interpretation of the data or in the decision to submit the manuscript for publication. None of the authors has declared any potential conflict of interest.

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doi:10.1016/j.jnutbio.2010.04.003

1. Introduction

Coronary heart disease (CHD) pathogenesis incorporates numerous interacting mechanisms being regulated by both genetic and environmental factors [1]. Among the environmental factors, in addition to tobacco smoking, physical activity and alcohol intake, dietary fat consumption, in particular, the amount of saturated fatty acids, has for many years been proposed as one of the main risk factors [2–4]. However, recent studies have questioned that general estimation by suggesting more specific effects of the amount of saturated fat for particular groups of individuals with certain genetic profiles [5–8].

Among the genetic determinants that have been more widely studied related to CHD risk is the common polymorphism in the apolipoprotein E (APOE) gene [9–11]. It has been widely shown that this polymorphism, involving codons 112 and 158, is a major determinant of plasma low-density lipoprotein cholesterol (LDL-C). Allele E3 is the most common one, E4 (rs 429358) is associated with increased LDL-C concentrations and E2 (rs 7412) is associated with decreased LDL-C [12]. Despite the consistent association of the E4 allele with higher LDL-C, the association of the *APOE* polymorphism with cardiovascular risk is more controversial [13]. There are various studies in which a greater risk of CHD has been found in carriers of the E4 allele than in E3/E3 subjects [9,10,14–17]. Nevertheless, other studies have not been able to find significant associations with a greater risk of CHD in E4 carriers [18–21]. Likewise, although some studies have associated the E2 allele with lower CHD risk [22–24], other studies have not found significant differences when comparing them with E3/E3 [10], and some studies have even attributed a greater CHD risk to E2 carriers under certain conditions [25,26]. This could be due to differences in study designs and even to the presence of random and systematic errors that may have an influence on those estimations [11]. However, the possible existence of gene–diet interactions in the different populations that modulate the genetic susceptibility conferred by those variants must not be overlooked. Hence, dietary fat, in particular, saturated fat intake, has been proposed as one of the main environmental factors that may interact with the *APOE* genotype modifying CHD risk [27–30]. Although some initial intervention studies have demonstrated that E4 carriers are more likely to respond to increased dietary fat intake with an increase of LDL-C [31,32], others failed to observe any difference [33,34]. This controversy has continued in later studies that analyzed the interaction between the *APOE* polymorphism and saturated fat intake in determining both LDL-C and CHD risk. In addition, a possible interaction of the *APOE* polymorphism with alcohol consumption in determining LDL-C [35] and high-density lipoprotein cholesterol (HDL-C) [36] has been described, but its potential influence on CHD risk is unknown. Therefore, our aims were (1) to examine the modulation that dietary fat and alcohol consumption have on the association between the *APOE* genotypes and LDL-C and HDL-C concentrations in a Mediterranean population, and (2) to study the association between the *APOE* polymorphism and incidence of CHD in this Mediterranean population by analyzing the possible interaction of the *APOE* polymorphism with saturated fat as well as with alcohol consumption.

2. Subjects and methods

2.1. Study design and recruitment

We performed a nested case-control study among participants of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study [37]. Cases were participants who developed an incident CHD event during follow-up, and controls were healthy matched subjects. EPIC is a large prospective study conducted in 10 European countries, whose methodological details have been published previously [38,39]. This study makes use of data from the Spanish cohort of EPIC-Heart, the cardiovascular branch of the study [40]. The population of the Spanish branch of EPIC included 41 440 individuals. Participants were healthy men (n=15 632) and women (n=25 806) volunteers, principally blood donors, aged 30 to 69 years at enrolment. They were recruited between October 1992 and July 1996 in five Spanish regions, three in Northern Spain (Asturias, Navarra and Gipuzkoa) and two in Southern Spain (Murcia and Granada). The cohort was followed up until December 2004; the mean follow-up period was ≈10 years. At recruitment, all participants gave their informed consent concerning the use of patient identifiable information. The study was approved by the ethical review boards.

2.2. Case ascertainment

Cases were defined as participants who experienced a definite CHD event (fatal or nonfatal myocardial infarction or angina requiring a revascularization procedure) during follow-up. Participants who at recruitment had a prior diagnosis of CHD that was validated thereafter were excluded from further analyses (n=193). For the identification of potential cases, a record linkage between the EPIC database and local hospital discharge registers was performed. A Population Myocardial Infarction Register, available in three participating regions (Navarra, Gipuzkoa and Murcia), was also used. Medical records of all potential cases were reviewed by trained personnel using the American Heart Association guidelines [41] for case validation. Until the end of 2004, a total of 719 potential cases were identified. Afterward, 459 definite cases of incident fatal and nonfatal myocardial infarction, 141 cases of angina requiring a revascularization procedure and 119 probable or possible myocardial infarction were classified from the entire cohort. Two controls were matched to each case by center, sex, age (within 5 years), and time of enrolment (within 3 months). In this study, we included 534 definite cases [nonfatal acute myocardial infarction (n=405), extra-hospitalized fatal acute myocardial infarction (n=8) and angina pectoris requiring revascularization procedure (n=121)] and 1123 healthy controls for whom buffy coat samples were available and it was possible to isolate DNA and determine the APOE genotype. Initially, controls were randomly selected among subjects in the cohort still at risk of CHD at the time of diagnosis of each potential case, and then, we considered as healthy controls those subjects who remained free of CHD after a the 10-year follow-up period, regardless of whether the case for which they were selected was probable or possible.

2.3. Demographic, clinical and lifestyle questionnaires

In face-to face interviews, each participant was administered questionnaires to collect information on sociodemographic characteristics; lifestyle factors, including food consumption and lifetime history of tobacco use; and medical history, including a prior diagnosis of hypertension, hyperlipidemia, diabetes mellitus or cardiovascular disease. Anthropometric measurements [height and weight, with calculation of body mass index (BMI) in kg/m2, and waist circumference] were obtained using standardized procedures. Diabetes status, hyperlipidemia and hypertension were self-reported. Physical activity was assessed as a part of the EPIC standardized lifestyle questionnaire, and its validity has been assessed [42]. Four categories were considered: inactive, moderately inactive, moderately active and active.

2.4. Dietary information

Information on usual food intake was collected by a validated computerized diet history questionnaire [43,44]. The validated dietary questionnaire was open but structured by meals (breakfast, mid-morning, lunch, mid-afternoon, dinner and after dinner) and included a list of around 600 common foods and recipes from each region. The frequency and amount of food consumed at least twice a month was recorded, taking seasonal variability into account. Portion intake of each food (in g/day) was quantified using household measures, standard units and a collection of 35 sets of photos of simple foods, mixed foods and drinks. The final amount of each food consumed was reported as daily intake (in grams). Energy and nutrient intakes were estimated using a conversion table in a computerized database especially compiled for the EPIC study in Spain [45]. Alcohol intake was considered both as two (drinker and nondrinker) or three categories: no drinker (0 g/d), moderate intake (<26.4 g alcohol/day for men and <13.2 g/day for women) and high intake (26.4 g alcohol/day for men and 13.2 g alcohol/day for women). These gram amounts correspond to 1 drink/day for women and 2 drinks/day for men [46]. Fat consumption variables were expressed in percentage of energy provided by this macronutrient. Two categories for total fat intake and for saturated fat intake were considered based in the most widely used international cutoff points for total fat consumption (greater or lesser than 30% of energy) and for saturated fat (greater or lesser than 10% of energy). For saturated fat, given that that cutoff value was similar to the population means, it was directly employed. For total fat, taking into account its high consumption in this Mediterranean population, more than 80% of subjects were consuming more than 30% of energy from fat, and two categories based on the population mean (greater or lesser than 36% energy) were considered. When food consumption variables were used in a continuous way, two individuals with very high energy intake (more than three S.D.'s) were excluded.

2.5. Biochemical determinations

Blood samples were taken by venipuncture into plain and citrate tubes at baseline and were divided into 0.5-ml aliquots of plasma, serum and buffy coat and stored in liquid nitrogen tanks at -196°C until assay. Close to 60% of blood samples were collected after an overnight fast. Lipid concentrations were measured on frozen samples in a central laboratory. We obtained data of plasma lipids for a random sample (one in two) of these cases and controls participating in a substudy aimed to measure plasma phytosterols. Thus, plasma total cholesterol and lipoproteins were successfully determined in 782 individuals. Cholesterol and triglycerides were analyzed by enzymatic procedures; triglycerides were measured only in fasting samples (n=477). HDL-C was quantified after precipitation with phosphotungstic acid and magnesium chloride. The concentration of LDL-C was calculated as total cholesterol minus HDL-C minus triglycerides/5 when triglyceride levels were ≤300 mg/dl in fasting samples, and by the homogeneous method of Daiichi Pure Chemicals (N-geneous LDL; Genzyme Diagnostics, Cambridge, MA) when triglyceride levels were >300 mg/dl and in nonfasting specimens. The determinations were made in an ADVIA 1800 chemical analyzer (Siemens Healthcare Diagnostics, Madrid, Spain). These determinations were expressed as corrected values taking into account the data of our previous study in which total cholesterol and triglycerides were determined in serum samples [47].

2.6. APOE genotyping

Genomic DNA was extracted from buffy coat with the MagNaPure LC DNA Isolation Kit (Roche Diagnostics, Mannheim, Germany). APOE genotyping was carried out by a validated single-tube protocol using fluorescent probes in the LightTyper instrument (Roche), as previously reported [48]. All genotyping was carried out blind at the Genetic and Molecular Epidemiology Unit, Valencia, Spain. Quality control procedures including positive and negative controls as well as replication of a random 15% of samples were applied. The duplicate concordance rate was higher than 98%.

2.7. Statistical analysis

Baseline characteristics were compared between future CHD cases and controls. χ^2 tests were used to test differences between observed and expected genotype frequencies, assuming Hardy–Weinberg equilibrium, and to test differences in percentages. Triglycerides were log transformed and alcohol intake was square root transformed for the statistical analyses. t and analysis of variance (ANOVA) tests were applied to compare crude means of demographic, anthropometric, dietary and biochemical variables between cases and controls and among the APOE genotypes. Multivariate adjustments of the association between the APOE polymorphism and plasma lipids were carried out by analysis of covariance, and adjusted means were estimated. Models were adjusted for sex, age, center, BMI (as continuous), fasting status, diabetes, hypertension, tobacco smoking (never, former, current smoker), alcohol consumption (yes, no), physical activity (inactive, moderately inactive, moderately active, and active), saturated fat (as dichotomic) and total energy intake (as continuous). The interaction term between the APOE polymorphism and dietary fat (total fat or saturated fat as dichotomous) or alcohol consumption (as dichotomous) on determining LDL-C and HDL-C was estimated in separated multivariate regression models including the corresponding main effects and interaction terms in addition to the above variables to control for. Stratified analyses were also carried out.

To test the hypothesis of association between the APOE polymorphism and incident CHD, unconditional logistic regression models were fitted. Several multivariate regression models were adjusted as detailed in results. Analyses were performed both by considering E2 carriers as the reference category and also the more prevalent E3/E3 genotype as the reference category. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated for the variant categories compared to the genotype group that was set as the reference category. Statistical analyses were performed with the SPSS package, version 15.0 (SPSS, Chicago, IL). All tests were two tailed, and P values <.05 were considered statistically significant.

3. Results

All subjects (incident CHD cases and controls) were free of CHD at baseline. Table 1 presents demographic, anthropometric, clinical, biochemical, dietary, lifestyle and genetic characteristics of incident CHD cases (n=534) and healthy controls (n=1123) at baseline. At recruitment, subjects in whom CHD developed during follow-up had higher concentrations of total cholesterol, LDL-C and triglycerides and lower HDL-C concentrations than controls, revealing an important contribution of plasma lipids in determining CHD risk in this Mediterranean population. Prevalence of drinkers was statistically lower in cases than in controls. No differences in the percentage of subjects consuming more than 10% of energy from saturated fat was found in future CHD cases and controls (P=.481). Both in cases and controls, the distribution of the APOE genotype frequencies observed did not deviate from those expected according to Hardy–Weinberg equilibrium (P=.226 and P=.169, respectively). On comparing the six APOE genotypes between cases and controls, no statistically significant differences were obtained (P=.302). On grouping the APOE genotypes in three categories (E2 carriers, E3/E3 and E4 carriers), although the frequency of E2 carriers was lower in cases (6.5%) than in controls (9.6%), we did not find statistical significant differences in the percentages comparison (P=.081). We excluded the 23 subjects with the mixed E2/E4 genotype for this and for further analyses. The APOE polymorphisms were highly associated with the variable indicating the self-reported hyperlipidemic status at baseline (P<.001).

Table 1
Baseline characteristics of CHD cases and control subjects

	CHD cases (n=534)	Controls (n=1123)	P
	Mean, S.D.	Mean, S.D.	
Men/women	424/110	884/239	.751
Age (years)	54.0 (7.3)	53.7 (7.2)	.467
BMI (kg/m^2)	29.2 (3.5)	28.7 (3.7)	.007
Total cholesterol (mg/dl)[a]	236.8 (37.0)	220.0 (34.3)	<.001
LDL-C (mg/dl)[a]	157.9 (34.6)	142.4 (32.1)	<.001
HDL-C (mg/dl)[a]	49.0 (14.8)	53.4 (13.6)	<.001
Fasting triglycerides (mg/dl)[b]	159.9 (118.3)	121.7 (73.7)	<.001
Energy intake (kcal/day)	2458.1 (776.3)	2516.0 (759.9)	.150
Total fat intake (g/day)	97.8 (36.2)	101.8 (36.2)	.034
Total fat intake (% energy)	35.7 (5.9)	36.3 (6.1)	.066
Saturated fat intake (g/day)	97.8 (36.2)	101.8 (36.2)	.028
Saturated fat (% energy)	10.5 (2.8)	10.9 (2.9)	.029
Monounsaturated fat (% energy)	15.1 (3.7)	15.7 (3.7)	.002
Polyunsaturated fat (% energy)	6.3 (2.6)	6.0 (2.5)	.090
Alcohol intake (g/day)	24.9 (32.0)	27.0 (32.3)	<.001
Alcohol intake in drinkers (g/day)	33.4 (33.1)	32.3 (32.8)	.555
Drinker status			<.001
Nondrinker, n (%)	136 (25.5)	184 (16.4)	
Moderate, n (%)	206 (38.6)	491 (43.7)	
High, n (%)	192 (36.0)	448 (39.9)	
Fat intake >30%, n (%)	442 (82.8)	960 (85.5)	.153
Saturated fat intake >10%, n (%)	295 (55.2)	641 (57.1)	.481
Smoking, n (%)			<.001
Current smokers	256 (48.0)	379 (33.8)	
Past smokers	106 (19.9)	255 (22.7)	
Never smokers	171 (32.1)	488 (43.5)	
Physical activity, n (%)			.183
Inactive	164 (31.4)	302 (27.8)	
Moderately inactive	173 (33.1)	338 (31.1)	
Moderately active	113 (21.6)	266 (24.5)	
Active	73 (14.1)	181 (16.7)	
Diabetes, n (%)	56 (10.5)	80 (7.1)	.021
Hypertensive, n (%)	186 (34.8)	263 (23.5)	<.001
Hyperlipidemic, n (%)	216 (43.8)	277 (24.7)	<.001
APOE genotype, n (%)			.302
E2/E2	1 (0.2)	1 (0.1)	
E2/E3	33 (6.2)	105 (9.3)	
E2/E4	7 (1.3)	16 (1.4)	
E3/E3	400 (74.9)	828 (73.7)	
E4/E3	91 (17.0)	167 (14.9)	
E4/E4	2 (0.4)	6 (0.5)	

P values for differences between cases and controls were obtained by unpaired t test for continuous variables and by χ^2 tests for categorical variables.
[a] Measured in 782 random samples (275 from cases and 507 from controls).
[b] Measured in 477 fasting samples.

3.1. Association between the APOE polymorphism and plasma lipid concentrations

Table 2 shows means of plasma cholesterol, LDL-C, HDL-C and triglycerides at baseline in a random sample (768, after exclusion of 14 E2/E4 subjects) of cases and controls by the APOE polymorphism. As expected, the most specific association of this polymorphism was

Table 2
Association between the APOE polymorphism and plasma lipids in incident CHD cases and controls at baseline

	CHD cases (n=272)		Controls (n=496)	
	n	Mean, S.D.	n	Mean, S.D.
Total cholesterol				
E2 carrier	18	221.0 (36.3)	42	203.6 (32.5)
E3/E3	205	235.7 (36.7)	370	220.8 (34.4)
E4 carrier	49	247.4 (36.8)	84	225.9 (32.9)
	P1=.023		P1=.002	
	P2=.010		P2=.001	
LDL-C				
E2 carrier	18	138.5 (39.1)	42	122.8 (34.2)
E3/E3	205	156.5 (33.1)	370	143.6 (31.6)
E4 carrier	49	171.4 (35.6)	84	148.6 (29.7)
	P1=.001		P1=<.001	
	P2=.001		P2=<.001	
HDL-C				
E2 carrier	18	46.3 (9.5)	42	56.8 (15.4)
E3/E3	205	49.6 (15.1)	370	53.6 (13.7)
E4 carrier	49	47.8 (15.6)	84	50.2 (12.6)
	P1=.558		P1=.025	
	P2=.708		P2=.010	
Triglycerides[a]				
E2 carrier	11	167.8 (57.7)	26	107.5 (89.0)
E3/E3	131	155.3 (121.0)	218	120.7 (68.8)
E4 carrier	30	171.0 (124.9)	52	134.1 (88.3)
	P1=.320		P1=.100	
	P2=.623		P2=.032	

P1: P value obtained in the global ANOVA test; P2: P value obtained in the test for lineal trend. Further adjustment of these comparisons by sex, age, center, fasting status, BMI, diabetes, hypertension, tobacco smoking, alcohol consumption, saturated fat, energy intake and physical activity.

[a] Triglycerides were measured in 463 fasting subjects. Total cholesterol, LDL-C and HDL-C were determined in fasting and nonfasting subjects in a random sample of cases and controls.

with LDL-C concentrations in both incident CHD cases and controls. LDL-C linearly increased from E2 to E4 carriers. A further adjustment of these associations by other confounding variables including the fasting and nonfasting status did not change the statistical significance of the results (not shown).

3.2. Interaction between the APOE polymorphism and diet in determining LDL-C and HDL-C

We studied the potential interaction between the APOE polymorphism and dietary fat or alcohol consumption in determining LDL-C and HDL-C concentrations. Only healthy controls were considered in these analyses. No statistically significant interactions with saturated fat intake were observed (Table 3). Although the interaction term between the *APOE* polymorphism and saturated fat in determining LDL-C was nonsignificant (P=.683), in the low saturated fat stratum (<10%), the effect of the *APOE* polymorphism was attenuated (P=.06), whereas in the high fat strata, mean LDL-C differences among the *APOE* genotypes were higher (P<.001). No significant effects were detected for HDL-C. When total fat intake (< or ≥36%) was considered, we did not find any significant interaction between this variable and the *APOE* polymorphism in determining LDL-C or HDL-C concentrations (results not shown).

However, in the case of alcohol consumption, we found an important modification of the effect of the polymorphism on LDL-C concentrations in control subjects depending on the drinker status (Fig. 1A). According to previously published results in the Framingham study, we observed that alcohol consumption in E2 subjects decreased LDL-C concentrations. However, in carriers of the E4 allele, alcohol consumption increased LDL-C concentration. This interaction was borderline significant in both men and women (P=.090) but reached the statistical significance in men (P for interaction=0.030).

Table 3
Association between the APOE polymorphism and LDL-C and HDL-C concentrations at baseline depending on the saturated fat intake (< or ≥10% of energy)

	Saturated fat <10%		Saturated fat ≥10%		P interaction
	n	Mean[a], S.E.	n	Mean, S.E.	
LDL-C					.969
E2 carrier	20	119.4 (10.0)	22	111.3 (10.0)	
E3/E3	164	137.5 (7.6)	206	133.7 (7.6)	
E4 carrier	36	139.9 (8.6)	48	141.6 (8.6)	
	P1=.061		P1=<.001		
HDL-C					.296
E2 carrier	20	53.4 (3.9)	22	54.2 (3.9)	
E3/E3	164	50.1 (2.9)	206	50.5 (2.8)	
E4 carrier	36	51.5 (3.9)	48	48.1 (3.3)	
	P1=.128		P1=.260		

Multivariate adjusted means in control subjects.
P1: P value obtained in the multivariate model for the APOE polymorphism adjusted for each stratum; P2: for interaction obtained for the APOE polymorphism and saturated fat in the multivariate adjusted models.

[a] Means were adjusted for sex, age, center, fasting status, BMI, diabetes, hypertension, tobacco smoking, alcohol consumption, energy intake and physical activity.

In both men and women, the interaction term between the APOE polymorphism and alcohol in determining LDL-C was statistically significant (P=.020) if two categories of the APOE polymorphism (E2 carriers and E4 carriers) were considered.

In terms of HDL-C (Fig. 1B), although we did not find a statistically significant interaction between the APOE polymorphism and alcohol consumption, we observed that in E2 subjects, alcohol consumption was associated with a greater increase in HDL-C concentrations. Moreover, in nondrinkers, the APOE polymorphism was not associated with HDL-C concentrations. However, in drinkers, the APOE polymorphism was associated with HDL-C (P=.013) with a decreasing effect from E2 to E4.

3.3. Association between the APOE polymorphism and CHD risk: modulation by fat and alcohol

Finally, the CHD risk associated with the APOE genotype was estimated in this Mediterranean population (Table 4). First, we adjusted a crude model in which only the APOE polymorphism, sex, age and center (Model 1) were included. In this model, E2 carriers were first considered as the reference category, and the risk of the other categories was estimated in comparison with it. A great risk of CHD was observed in both E3/E3 and E4 carriers. This protective effect of the E2 allele in comparison with the other genotypes remained significant after additional adjustment for BMI, tobacco smoking, alcohol drinking, diabetes and hypertension (Model 2). A later additional adjustment for saturated fat, energy and physical activity did not alter the statistical significance of the estimations. However, on considering E3/E3 subjects as the reference category, a lower risk of CHD associated with the E2 allele was observed in Model 2 and in Model 3. Nevertheless, the greater risk detected for E4 carriers did not reach statistical significance.

On studying whether this association of the APOE polymorphism with CHD risk was modified by a high or low saturated fat intake (Table 5), it was observed that saturated fat modulated that association in such a way that in the low fat intake stratum (<10% of energy), the E3/E3 and E4 carriers did not present significant differences to the E2 carriers in terms of CHD risk. However, with a high saturated fat intake (≥10% of energy), the CHD risk associated with the E3/E3 subjects, and in particular, with E4 carriers, was magnified in comparison with E2 carriers. This CHD risk in E4 carriers with a high level of saturated fat intake, despite increasing in magnitude, did not reach the statistical significance when compared

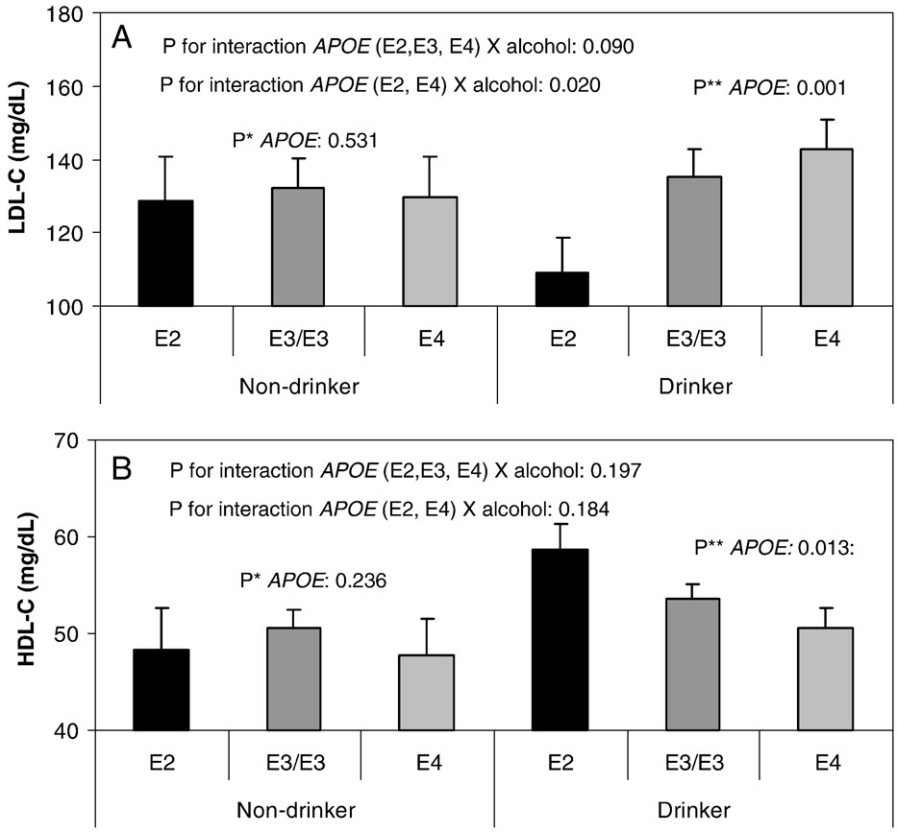

Fig. 1. Plasma LDL-C (A) and HDL-C (B) concentrations depending on the APOE polymorphism and alcohol consumption in control subjects (n=496). Multivariate adjusted means are estimated by separated regression models containing main effects and the interaction term between the *APOE* polymorphism (expressed as three categories as well as with two extreme categories) and adjustment for covariates. Models were adjusted for sex, age, center, fasting state, BMI, diabetes, hypertension, tobacco smoking, alcohol consumption, physical activity, saturated fat and total energy intake. The statistical significance of interaction terms in the corresponding multivariate models is indicated. *P value for the APOE polymorphism in the stratified multivariate model in nondrinkers. **P value for the APOE polymorphism in the stratified multivariate models in drinkers. Error bars: S.E.M.

to the E3/E3 reference category. This can be attributed to a decreased statistical power after stratification.

The potential interaction between alcohol consumption and the APOE polymorphism in determining CHD risk was also studied (Table 6). In the nondrinker category, the APOE polymorphism was not significantly associated with CHD risk (P=.507). However, in drinkers, E2 carriers did present significantly lower CHD risk, with the risk in E4 carriers being higher. If instead of two categories of alcohol consumption, three strata are considered, it is observed that the decrease in CHD risk in E2 carriers associated with alcohol consumption was magnified in the highest stratum (OR for E2 carriers compared with E3/E3 in the high alcohol consumption stratum: 0.45; P=.032).

4. Discussion

In this nested case-control population-based study carried out on the Spanish EPIC cohort, we have found a strong association between the *APOE* genotype and plasma LDL-C concentrations in both CHD cases and controls at baseline. These results are similar to those

Table 4
Association between the APOE polymorphism and incident CHD in the Mediterranean population

	n	Model 1[a]		Model 2[b]		Model 3[c]	
		OR, 95% CI	P	OR, 95% CI	P	OR, 95% CI	P
CHD risk							
E2 carrier	140	1.00 (reference)		1.00 (reference)		1.00 (reference)	
E3/E3	1228	1.50 (1.00–2.24)	.050	1.63 (1.12–2.64)	.022	1.72 (1.00–2.24)	.013
E4 carrier	266	1.67 (1.05–2.65)	.029	1.92 (1.24–3.31)	.007	2.02 (1.05–2.65)	.005
		P1=.087		P1=.027		P1=.018	
CHD risk							
E2 carrier	140	0.67 (0.45–1.00)	.050	0.62 (0.41–0.93)	.022	0.58 (0.38–0.89)	.013
E3/E3	1228	1.00 (reference)		1.00 (reference)		1.00 (reference)	
E4 carrier	266	1.12 (0.84–1.48)	.445	1.18 (0.88–1.58)	.263	1.17 (0.88–1.58)	.280

Logistic regression analysis.
P1: P value obtained for the global APOE polymorphism in the multivariate logistic regression model.
[a] Model adjusted for sex, age, center.
[b] Model adjusted for sex, age, center, BMI, diabetes, hypertension, tobacco smoking and alcohol consumption.
[c] Model adjusted for sex, age, center, BMI, diabetes, hypertension, tobacco smoking, alcohol consumption, saturated fat, total energy intake and physical activity.

Table 5
Association between the APOE polymorphism and incident CHD depending on the saturated fat intake (greater or lesser than 10% of energy)[a]

	Low saturated fat (<10%)			High saturated fat (>10%)		
	n	OR, 95% CI	*P*	*n*	OR, 95% CI	*P*
CHD risk						
E2 carrier	66	1.00 (reference)		74	1.00 (reference)	
E3/E3	512	1.22 (0.67–2.22)	.509	716	2.61 (1.36–4.97)	.004
E4 carrier	134	1.35 (0.69–2.67)	.383	132	3.33 (1.61–6.90)	.001
	P1=.683			P1=.005		
CHD risk						
E2 carrier	66	0.82 (0.45–1.48)	.509	74	0.38 (0.20–0.73)	.004
E3/E3	512	1.00 (reference)		716	1.00 (reference)	
E4 carrier	134	1.11 (0.72–1.70)	.644	132	1.28 (0.85–1.93)	.240

Stratified logistic regression analysis.
P1: *P* value obtained for the global APOE polymorphism in the multivariate logistic regression model.
[a] Models adjusted for sex, age, center, BMI, diabetes, hypertension, tobacco smoking, alcohol consumption, total energy intake and physical activity (Model 3).

obtained in multiple populations being estimated in a metaanalysis in 86 067 individuals [13]. Despite the huge controversy existing in the results published in individual studies on the higher or lower CHD risk for the E2 and E4 alleles, in this metaanalysis [13], it was estimated that E2 carriers had a lesser risk of having CHD than E3/E3 subjects (OR, 0.80; 95% CI, 0.70–0.90). They also found that the E4 carriers had a slightly higher risk of CHD than E3/E3 did, without reaching the statistical significance (OR, 1.06; 95% CI, 0.99–1.13). Accordingly, in the present study, we found a clear lower CHD risk in E2 carriers in comparison to E3/E3, and we did not find a significant higher risk in E4 carriers compared with E3/E3.

Our study has the additional advantage that incident CHD cases have been identified prospectively over a 10-year period, thus, minimizing the likelihood of bias that other study designs that consider prevalent cases have. Moreover, confirmation of CHD cases has followed rigorous international criteria, also increasing the validity of the estimations. It is also the first study in which the association between the *APOE* polymorphism and incidence of CHD has been investigated in the Spanish Mediterranean population.

Although the association between the E4 allele and higher LDL-C concentrations is very consistent in different studies, the association between the E4 allele and CHD is controversial. Despite the fact that some initial studies showed a significant association with higher CHD, later studies have not been able to replicate those findings. Thus, in the metaanalysis undertaken by Bennet et al. [11], a great heterogeneity between studies was found for this association. They concluded that the association between the E4 allele and CHD differs according to sample size of the studies considered. Hence, in studies that include at least 500 cases of CHD, the E4 allele is not significantly associated with CHD risk, whereas the E2 allele is significantly associated with a lower CHD risk. These estimations were similar for men and women, for people older or younger than 55 years and in case-control vs. cohort studies. By contrast, on considering studies with less than 500 CHD cases analyzed, the E4 allele was significantly associated with higher CHD risk, whereas for the E2 allele, a lower significant risk was not found. These differences are not easy to explain, perhaps being attributable to publication bias at a time when it was of interest to stress the greater CHD risk associated with the E4 allele. Hence, if the presence of some environmental interaction (i.e., dietary fat, alcohol consumption, tobacco smoking) boosted the harmful effect of the E4 allele on a certain population, obtaining under these conditions statistically significant differences in the estimated risk, it was more likely for those effects to be published even if that study was of small size.

Table 6
Association between the APOE polymorphism and incident CHD depending on the drinker status (drinker and nondrinkers)[a]

	Nondrinker			Drinker		
	n	OR, 95% CI	*P*	*n*	OR, 95% CI	*P*
CHD risk						
E2 carrier	36	1.00 (reference)		104	1.00 (reference)	
E3/E3	239	1.62 (0.72–3.67)	.245	989	1.76 (1.05–2.94)	.032
E4 carrier	40	1.58 (0.57–4.42)	.381	226	2.18 (1.23–3.87)	.008
	P1=.507			P1=.028		
CHD risk						
E2 carrier	36	0.62 (0.27–1.39)	.245	104	0.57 (0.34–0.95)	.032
E3/E3	239	1.00 (reference)		989	1.00 (reference)	
E4 carrier	40	0.97 (0.47–2.05)	.947	226	1.24 (0.90–1.71)	.192

Stratified logistic regression analysis.
P1: *P* value obtained in for the global APOE polymorphism in the multivariate logistic regression model.
[a] Models adjusted for sex, age, center, BMI, diabetes, hypertension, tobacco smoking, saturated fat, total energy intake and physical activity (Model 3).

In our study, we have observed an interaction between saturated fat and the APOE genotype in determining CHD risk in such a way that the general estimation of a greater CHD risk in E4 carriers compared to E2 carriers does not reach statistical significance when the contribution of saturated fat in the diet was lower than 10% of energy intake. In the same way, no statistical significance of a greater CHD risk was reached in the E3/E3 subjects compared to E2 carriers in this stratum. Nevertheless, when saturated fat is greater than 10% of energy, the differences are magnified, with a higher CHD risk being observed in E4 carriers in comparison to E2 carriers. No statistically significant differences in comparison with E3/E3 were observed because of the limited power of our study after stratification. Higher CHD risk of the E4 allele increased by a high saturated fat intake has also been observed in a case-control study carried out in Costa Rica [30]. However, in contrast to our results, they found that a high saturated fat consumption significantly increased the risk of myocardial infarction both in E4 carriers and in E2 carriers. Furthermore, they observed that a higher saturated fat intake increased LDL-C concentrations both in E2 and E4 carriers. In our study, despite that we did not find an interaction between saturated fat and the APOE polymorphism on determining LDL-C concentrations, we observed that higher saturated fat intake magnified the genotypic effect on LDL-C means. On the other hand, the results of our study allow us to hypothesize that the greater CHD risk in *APOE4* carriers in a high saturated fat consumption situation, compared to E2 carriers, could involve mechanisms other than the greater increase of LDL-C presumably attributed to that interaction. Furthermore, it should be borne in mind that the interaction between the APOE genotype and saturated fat intake in determining LDL-C concentrations has been measured cross-sectionally, whereas the interaction between the *APOE* genotype and saturated fat intake in determining cardiovascular risk has been estimated longitudinally, with a possible meaning that other mechanisms (LDL size, LDL oxidation, HDL concentrations and oxidation, increases in VLDL, postprandial effects, etc.), which are becoming stronger over time, are involved. The effect of the saturated fat increasing CHD risk in E4 carriers is observed to be stronger when comparing E4 and E2 carriers, which are those who have greater differences in protein function.

Another interesting interaction that we have found in our study is that involving alcohol consumption. Previous studies have shown that alcohol consumption in E2 carriers reduces LDL-C concentrations [35,49]. On the other hand, alcohol consumption in E4 carriers would not be favorable given that it would increase LDL-C [35]. In this study, we have replicated this interaction and obtained consistent results in terms of CHD risk. Moreover, we have found that alcohol consumption largely increases HDL-C concentrations in E2 subjects, and in drinkers, E4 subjects have the lower HDL-C concentrations. This

interaction is in accordance with that previously described in North Americans [36]. Furthermore, the protective effect of the E2 allele on CHD in comparison with E3/E3 subjects was not significant in nondrinkers. In this stratum, no greater risk of CHD was observed in E4 carriers compared with E2 subjects. In contrast, in drinkers, carriers of the E2 allele did present statistically lower risk of CHD compared to E3/E3. Few other studies have analyzed the interaction between alcohol consumption and the APOE polymorphism on CHD risk having observed different effects. Mukamal et al. [50] in the Cardiovascular Health Study, a prospective cohort study of community-dwelling older adults in the United States, did not find a greater CHD risk in E4 carriers who consumed alcohol, and they reached the conclusion that the effect of alcohol intake increasing CHD risk in E4 carriers would be more specific for young adults for which HDL-C concentrations are more relevant. In our study, the mean age of the subjects was not very high, increasing therefore the likelihood of detecting these effects.

In conclusion, in this nested case-control study carried out on a Mediterranean population, we have found a strong association between the APOE polymorphism and LDL-C concentrations as well as a dietary modulation in determining CHD risk. In addition to their lower LDL-C concentrations, E2 carriers have a lower incidence of CHD than E3/E3 subjects, with the greater CHD risk in E4 carriers not reaching the statistical significance compared to E3/E3, although it does so in comparison with E2 carriers. Moreover, we have observed that saturated fat intake modulates CHD risk in such a way that in the lower consumption stratum (<10% of energy), the genetic effect was not observed, whereas in the higher consumption stratum, the differences in CHD risk between E2 and E4 carriers were magnified. Our results also suggest that moderate alcohol consumption may be more favorable in E2 carriers both on plasma lipids and CHD risk.

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