

# Alcohol drinking determines the effect of the *APOE* locus on LDL-cholesterol concentrations in men: the Framingham Offspring Study<sup>1-3</sup>

Dolores Corella, Katherine Tucker, Carlos Lahoz, Oscar Coltell, L Adrienne Cupples, Peter WF Wilson, Ernst J Schaefer, and Jose M Ordovas

See corresponding editorial on page 669.

## ABSTRACT

**Background:** The effect of alcohol drinking on LDL-cholesterol concentrations is unclear. The reported variability may be due to interactions between genetic factors and alcohol intake.

**Objective:** The purpose of the study was to examine whether variation at the apolipoprotein E gene (*APOE*) locus modulates the association between alcohol drinking and LDL cholesterol.

**Design:** We used a cross-sectional design in a healthy population-based sample of 1014 men and 1133 women from the Framingham Offspring Study.

**Results:** In male nondrinkers ( $n = 197$ ), LDL cholesterol was not significantly different across *APOE* allele groups [*APOE*\**E2* (*E2*), *APOE*\**E3* (*E3*), and *APOE*\**E4* (*E4*)]. However, in male drinkers ( $n = 817$ ), differences were observed ( $P < 0.001$ ); those with the *E2* allele had the lowest concentrations. LDL cholesterol in men with the *E2* allele was significantly lower in drinkers than in nondrinkers but was significantly higher in drinkers than in nondrinkers in men with the *E4* allele. This *APOE*-alcohol interaction remained significant ( $P < 0.001$ ) after age, body mass index, smoking status, and fat and energy intakes were controlled for. In women, the expected effect of *APOE* alleles on LDL cholesterol occurred in both drinkers ( $n = 791$ ;  $P < 0.001$ ) and nondrinkers ( $n = 342$ ;  $P < 0.001$ ). Multiple linear regression models showed a negative association ( $P < 0.05$ ) between alcohol and LDL cholesterol in men with the *E2* allele but a positive association in men with the *E4* allele. No significant associations were observed in men or women with the *E3* allele.

**Conclusion:** In men, the effects of alcohol intake on LDL cholesterol are modulated in part by variability at the *APOE* locus. *Am J Clin Nutr* 2001;73:736-45.

**KEY WORDS** Apolipoprotein E, genetics, lipoproteins, LDL cholesterol, alcohol drinking, diet, the Framingham Offspring Study, *APOE*

## INTRODUCTION

Genetic variation at the apolipoprotein E gene (*APOE*) locus is an important determinant of serum LDL-cholesterol concentrations. In general, carriers of the *APOE*\**E2* (*E2*) allele have lower LDL-cholesterol concentrations and carriers of the

*APOE*\**E4* (*E4*) allele have higher LDL-cholesterol concentrations than do homozygotes for the *APOE*\**E3* (*E3*) allele (1-4). However, these effects may be modulated by factors such as age, sex, ethnicity, and environmental conditions (5-7). Several gene  $\times$  diet (fat and cholesterol) interactions were reported to be associated with the variability in LDL-cholesterol response to dietary intervention (8, 9). Other interactions with behavioral factors, such as smoking and alcohol intake, have been explored less. The effect of alcohol intake on plasma lipids and coronary heart disease (CHD) has been the subject of extensive research. Both case-control and cohort studies have described a J- or U-shaped association between alcohol intake and CHD (10, 11) and between alcohol intake and all-cause mortality (12). Men and women with an intake of 1-2 drinks/d (moderate alcohol intake) appear to have the lowest risk of CHD (13-15). Experimental and observational studies reported that moderate alcohol intake is associated with increased HDL-cholesterol concentrations (16, 17). This information was summarized in a recent meta-analysis that concluded that alcohol intake is causally related to a lower risk of CHD because of changes in lipid and hemostatic factors (18). However, the effect of alcohol intake on LDL cholesterol is unclear and was not presented in this meta-analysis. In general, weak direct (19) or inverse (20, 21) associations between alcohol and LDL-cholesterol concentrations have been reported, as have no associations (22, 23). Interestingly, Hein et al (24) examined the connection between alcohol, LDL cholesterol, and risk of CHD in a cohort study and showed that the effect of alcohol on the risk of CHD depends on LDL-cholesterol

<sup>1</sup>From the Lipid Metabolism Laboratory and the Epidemiology Program, Jean Mayer-US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston; the Boston University School of Public Health, Boston; and the Boston University School of Medicine, Framingham, MA.

<sup>2</sup>Supported by grant HL54776 and contract NOI-38038 from the NIH/NHLBI and contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Agricultural Research Service.

<sup>3</sup>Address reprint requests to JM Ordovas, Lipid Metabolism Laboratory, USDA, HNRCA at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: ordovas@hnrc.tufts.edu.

Received May 3, 2000.

Accepted for publication October 17, 2000.

concentrations. The inconsistency of the results reported by different studies suggests that other factors, including genetic variability, may modify the association between LDL-cholesterol concentrations and alcohol intake. The purpose of this population-based study was to examine whether variation at the *APOE* locus modulates the association between alcohol drinking and LDL-cholesterol concentrations.

## SUBJECTS AND METHODS

### Subjects and study design

This study was carried out by using a cross-sectional design. The details of the design and methods of the Framingham Offspring Study are presented elsewhere (25). Starting in 1971, 5124 subjects were enrolled (26). Blood samples for DNA analysis were collected between 1987 and 1991. Lipid phenotypes, DNA, and information on CHD risk factors and diet were available for 1014 men and 1133 women who attended the fifth examination visit of the Framingham Offspring Study, conducted between 1992 and 1995; these participants were free of CHD (including myocardial infarction, angina pectoris, and coronary insufficiency), were not taking lipid-lowering medication, were not receiving estrogen replacement therapy, and had plasma triacylglycerol concentrations  $\leq 4.52$  mmol/L. Nearly all subjects were white. Data on smoking, height, and weight were obtained as described previously (26, 27). All procedures were approved by the institutional human investigation review boards.

### Plasma lipid and lipoprotein measurements

Twelve-hour fasting venous blood samples were collected in tubes containing 0.1% EDTA. Plasma was separated from blood cells by centrifugation ( $1600 \times g$ , 10 min,  $4^{\circ}\text{C}$ ) and was used immediately for lipid measurements. Plasma total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were measured as described previously (28). HDL cholesterol was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins with dextran sulfate–magnesium sulfate (29). LDL-cholesterol concentrations were estimated with the equation of Friedewald et al (30). CVs for total cholesterol, HDL-cholesterol, and triacylglycerol measurements were each  $< 5\%$  (31).

### DNA isolation and genotyping

Leukocyte DNA was extracted from 5–10 mL whole blood with the method described by Miller et al (32). *APOE* genotypes were determined as described by Hixson and Vernier (33). A 244-base pair fragment of the *APOE* gene, which included the 2 polymorphic sites, was amplified by polymerase chain reaction in a DNA Thermal Cycler (PTC-100; MJ Research, Inc, Watertown, MA) by using oligonucleotide primers F4 and F6. Each reaction mixture was heated at  $90^{\circ}\text{C}$  for 2 min, followed by 35 cycles of amplification ( $94^{\circ}\text{C}$  for 40 s,  $62^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 1 min). The polymerase chain reaction products were digested with 5 units of *HhaI* and the fragments were separated by electrophoresis in an 8%-polyacrylamide nondenaturing gel. After electrophoresis, the gel was treated with ethidium bromide for 30 min and the DNA fragments were visualized by using ultraviolet illumination.

### Dietary information

Dietary intake was estimated with the semiquantitative food-frequency questionnaire of Willett et al (34). This questionnaire

includes 136 food items, with questions concerning intakes of beer, wine, and spirits. Subjects were asked to report their frequency of use of each item per day, week, or month. Intake frequencies are linked with nutrient data at Harvard University to obtain estimates of daily intakes (35). We measured fat intake in absolute values (g/d) and as a percentage of total energy intake. Subjects were classified into 3 groups according to saturated fat intake: low ( $\leq 10\%$  of energy/d), moderate (10–14% of energy/d), and high ( $\geq 14\%$  of energy/d). Saturated fat was strongly correlated with total fat ( $r > 0.9$ ,  $P < 0.001$ ); therefore, total fat was not included in the multivariate models with saturated fat.

### Alcohol

We calculated alcohol intake (in g/d) for each individual on the basis of the type and amount of alcoholic beverages consumed in the previous year. Two different models were considered. In the first model, subjects were divided between non-drinkers (those who reported no intake of alcohol for several years before the examination) and drinkers (those who reported intake of any amount of alcohol). The second model tested dose-effect relations between alcohol and LDL-cholesterol concentrations. Three groups were defined according to the reported daily intake of alcohol: no intake (0 g/d), moderate intake ( $< 26.4$  g alcohol/d for men and  $< 13.2$  g/d for women), and high intake ( $\geq 26.4$  g alcohol/d for men and  $\geq 13.2$  g alcohol/d for women). These gram amounts correspond to  $\approx 1$  drink/d for women and  $\approx 2$  drinks/d for men. On the basis of epidemiologic studies with health outcomes, these amounts were defined by several organizations as the upper cutoff for moderate drinking (36).

### Statistical analyses

The SAS statistical package (version 6.12; SAS Institute Inc, Cary, NC) was used for the analyses. Triacylglycerol values were  $\log_{10}$  transformed and alcohol intake was square-root transformed to improve normality for statistical testing with continuous variables. To assess mean differences for continuous variables between sexes, Student's *t* test for independent groups was used. One-way analysis of variance (ANOVA) was used for multiple comparisons of means. *P* values for linear trends between categories were also calculated in the ANOVA by partitioning the between-group sums of squares into trend components. Bonferroni tests were applied to correct for multiple comparisons. Allele frequencies were estimated by gene counting. Chi-square tests were conducted to compare differences between observed and expected frequencies, assuming Hardy-Weinberg equilibrium (37), and to test for differences in percentages between groups. Pearson correlation coefficients were calculated to describe crude associations between total fat and saturated fat, dietary cholesterol, energy intake, and alcohol intake (g/d) in men and women by *APOE* allele type. Partial correlation coefficients were also estimated with adjustment for one or more confounding variables.

Analysis of covariance was carried out by using the general linear model procedure to evaluate the relation between LDL-cholesterol concentrations and categorical independent variables, with control for the effect of continuous independent variables, and to test the effect of interaction terms. We used analysis of covariance techniques that accounted for the familial relations among the study participants (mostly siblings and cousins). We used a repeated-measures approach (PROC MIXED; SAS

**TABLE 1**  
Demographic, biochemical, and dietary characteristics of the population

	Men ( <i>n</i> = 1014)		Women ( <i>n</i> = 1133)	
	Drinkers ( <i>n</i> = 817)	Nondrinkers ( <i>n</i> = 197)	Drinkers ( <i>n</i> = 791)	Nondrinkers ( <i>n</i> = 342)
Age (y)	54.0 ± 9.7 <sup>1</sup>	54.0 ± 10.1	53.4 ± 9.5	55.5 ± 9.8 <sup>2</sup>
Body mass index (kg/m <sup>2</sup> )	28.1 ± 4.0	28.1 ± 4.2	26.1 ± 5.3	27.5 ± 5.5 <sup>2</sup>
Total cholesterol (mmol/L)	5.23 ± 0.87	5.38 ± 0.97 <sup>2</sup>	5.33 ± 0.93	5.38 ± 0.97
LDL cholesterol (mmol/L)	3.34 ± 0.79	3.21 ± 0.80 <sup>2</sup>	3.17 ± 0.88	3.28 ± 0.84 <sup>2</sup>
HDL cholesterol (mmol/L)	1.17 ± 0.29	1.01 ± 0.25 <sup>2</sup>	1.53 ± 0.39	1.33 ± 0.35 <sup>2</sup>
Triacylglycerol (mmol/L)	1.58 ± 0.83	1.69 ± 0.91	1.37 ± 0.71	1.56 ± 0.75 <sup>2</sup>
Total fat (g/d)	65.9 ± 26.0	73.2 ± 30.1 <sup>2</sup>	56.7 ± 23.6	56.3 ± 22.5
Saturated fat (g/d)	23.7 ± 10.3	26.4 ± 11.5 <sup>2</sup>	20.1 ± 9.1	20.2 ± 8.7
Cholesterol (mg/d)	242.0 ± 90.2	205.5 ± 94.9	211.4 ± 90.3	205.5 ± 94.9
Energy intake (MJ/d) <sup>3</sup>	8.35 ± 2.64	8.68 ± 2.79	7.28 ± 2.40	7.08 ± 2.37
Alcohol (g/d)	19.0 ± 20.2	0.0 ± 0.0 <sup>2</sup>	15.5 ± 13.0	0.0 ± 0.0 <sup>2</sup>
Systolic blood pressure (mm Hg)	128 ± 16	129 ± 19	123 ± 19	125 ± 21
Diastolic blood pressure (mm Hg)	82 ± 10	81 ± 11	79 ± 10	77 ± 10
Current smokers [ <i>n</i> (%)]	146 (17.9)	43 (21.8)	150 (19.0)	70 (21.5)

<sup>1</sup> $\bar{x} \pm SD$ ; percentage in parentheses

<sup>2</sup>Significantly different from drinkers,  $P < 0.05$ .

<sup>3</sup>1 MJ = 239.23 kcal.

Institute Inc) that assumed an exchangeable correlation structure among all members of a family. To test the null hypothesis of no interactions between *APOE* allele groups and environmental factors, analysis of covariance was performed that included LDL-cholesterol concentration as the outcome variable and alcohol intake (2 categories) and *APOE* allele group (3 categories) as fixed factors (main effects and two-way interaction); age, BMI, and total energy intake as continuous covariates; and saturated fat intake (3 categories) and tobacco smoking (2 categories) as control factors.

To estimate the extent, direction, and strength of relations between LDL-cholesterol concentrations and several independent variables, with or without interaction terms, multiple linear regression analysis with dummy variables (for categorical and interaction terms) was applied. The interaction terms between alcohol intake dummies and *APOE* allele groups were created as two-factor products. Regression diagnostics (analysis of residuals, influence of outliers, and colinearity) were used to check the assumptions and to assess the accuracy of computations. The average effects of the 3 *APOE* allele groups on LDL-cholesterol concentrations in the whole male cohort and in alcohol drinking and nondrinking men were calculated by using the formula of Sing and Davignon (38).

## RESULTS

Demographic, anthropometric, dietary, and plasma lipid data for the study subjects (1014 men and 1133 women) by sex and alcohol intake status are summarized in **Table 1**. All variables, except age, LDL-cholesterol concentrations, and tobacco smoking, differed significantly between men and women. The genotype frequencies of the 3 *APOE* alleles (*E2*, *E3*, and *E4*) were not significantly different by sex and did not deviate significantly from those predicted by the Hardy-Weinberg equilibrium (37). The observed allele frequencies were 0.82, 0.11, and 0.071 for the *E3*, *E4*, and *E2* alleles, respectively.

Plasma lipid concentrations and dietary intakes by sex in carriers of the *E2* allele (*E2/E3* and *E2/E2* genotypes), those homozygous for the *E3* allele (*E3/E3* genotype), and carriers of

the *E4* allele (*E3/E4* and *E4/E4* genotypes) are shown in **Table 2**. Subjects with the *E2/E4* genotype (15 men and 18 women) were excluded from this analysis. *APOE* allele type had a significant effect on total and LDL-cholesterol concentrations in both men and women ( $P < 0.001$ ). Subjects with the *E2* allele had significantly lower total cholesterol and LDL-cholesterol concentrations than did subjects with the *E3* or *E4* allele. After Bonferroni correction, no significant differences in means were observed between subjects with the *E3* and *E4* alleles. Mean dietary intakes of total fat, saturated fat, cholesterol, and alcohol did not differ significantly between *APOE* allele types.

There were highly positive and significant Pearson correlation coefficients between saturated fat and cholesterol and energy intakes in both men and women but no significant differences in intakes by *APOE* allele type (**Table 3**). Alcohol intake showed no significant associations with nutrients and a weak positive association with energy intake. The highest correlation coefficient ( $r = 0.268$ ,  $P < 0.01$ ) for alcohol and energy intakes was observed in men with the *E2* allele.

More men (80.6%) than women (69.8%) drank alcohol ( $P < 0.001$ ) and more men (21.2%) than women (17.5%) had a high intake of alcohol ( $P < 0.001$ ). The percentage of current smokers was not significantly different between sexes (19% in both groups). Saturated fat intake, expressed as a percentage of energy intake, was lower in women than in men ( $P < 0.02$ ). In men, 41.2%, 45.7%, and 13.1% consumed low, moderate, and high amounts of saturated fat, respectively; in women, the corresponding values were 45.7%, 43.6%, and 10.7%, respectively.

In men, mean LDL-cholesterol concentrations were higher in drinkers ( $3.34 \pm 0.79$  mmol/L) than in nondrinkers ( $3.21 \pm 0.80$  mmol/L;  $P = 0.018$ ). Conversely, in women, mean LDL-cholesterol concentrations were higher in nondrinkers ( $3.28 \pm 0.84$  mmol/L) than in drinkers ( $3.17 \pm 0.88$  mmol/L) ( $P = 0.021$ ). Mean LDL-cholesterol concentrations in men and women according to *APOE* allele type and alcohol intake are shown in **Figure 1**. In male nondrinkers, mean LDL-cholesterol concentrations did not differ significantly across *APOE* allele types; the expected lowering effect of the *E2* allele was absent and the usual elevating effect of the *E4* allele was reversed. In male

TABLE 2

Plasma lipid concentrations, alcohol intake, tobacco smoking, and dietary fat intake by *APOE* allele type and sex

	<i>APOE</i> allele <sup>1</sup>			<i>P</i> <sup>2</sup>	<i>P</i> for trend <sup>3</sup>
	<i>E2</i> ( <i>n</i> = 125 M, 170 F)	<i>E3</i> ( <i>n</i> = 691 M, 736 F)	<i>E4</i> ( <i>n</i> = 198 M, 227 F)		
Age (y)					
Men	54.1 ± 9.4 <sup>4</sup>	54.2 ± 9.8	53.8 ± 9.9	0.856	0.783
Women	54.4 ± 9.4	54.1 ± 9.8	53.6 ± 9.1	0.671	0.399
BMI (kg/m <sup>2</sup> )					
Men	28.6 ± 4.0	28.0 ± 4.1	28.0 ± 4.0	0.856	0.783
Women	26.3 ± 5.5	26.4 ± 5.3	27.0 ± 5.8	0.671	0.399
Total cholesterol (mmol/L)					
Men	4.92 ± 0.96 <sup>5,6</sup>	5.22 ± 0.85 <sup>7</sup>	5.34 ± 0.97 <sup>7</sup>	<0.001	<0.001
Women	5.09 ± 1.06 <sup>5,6</sup>	5.38 ± 0.95 <sup>7</sup>	5.49 ± 0.91 <sup>7</sup>	<0.001	<0.001
LDL cholesterol (mmol/L)					
Men	2.92 ± 0.86 <sup>5,6</sup>	3.37 ± 0.77 <sup>7</sup>	3.43 ± 0.83 <sup>7</sup>	<0.001	<0.001
Women	2.88 ± 0.90 <sup>5,6</sup>	3.25 ± 0.85 <sup>7</sup>	3.35 ± 0.82 <sup>7</sup>	<0.001	<0.001
HDL cholesterol (mmol/L)					
Men	1.14 ± 0.32	1.14 ± 0.29	1.08 ± 0.28	0.043	0.066
Women	1.51 ± 0.43	1.47 ± 0.39	1.43 ± 0.40	0.101	0.032
Triacylglycerol (mmol/L)					
Men	1.99 ± 1.43 <sup>5,6</sup>	1.71 ± 1.22 <sup>7</sup>	1.87 ± 1.31 <sup>7</sup>	0.014	0.438
Women	1.51 ± 0.74	1.46 ± 0.87	1.61 ± 1.17	0.146	0.314
Total fat (g/d)					
Men	70.7 ± 27.0	66.7 ± 27.0	67.8 ± 28.0	0.293	0.329
Women	57.4 ± 22.5	56.7 ± 23.3	55.9 ± 23.7	0.815	0.526
Saturated fat intake (g/d)					
Men	25.8 ± 10.8	24.0 ± 10.5	24.0 ± 10.9	0.212	0.137
Women	20.0 ± 8.5	20.2 ± 9.0	20.0 ± 9.5	0.950	0.980
Cholesterol intake (mg/d)					
Men	250.9 ± 93.0	243.7 ± 116.8	249.3 ± 113.5	0.709	0.895
Women	218.2 ± 101.3	209.5 ± 91.3	203.4 ± 85.0	0.279	0.110
Energy intake (MJ/d) <sup>8</sup>					
Men	8.7 ± 2.8	8.4 ± 2.7	8.4 ± 2.7	0.626	0.438
Women	7.3 ± 2.3	7.3 ± 2.4	7.1 ± 2.4	0.508	0.286
Alcohol intake (g/d)					
Men	13.3 ± 17.9	15.6 ± 19.9	16.0 ± 20.5	0.398	0.551
Women	8.1 ± 12.3	6.7 ± 11.3	8.7 ± 13.3	0.063	0.603
Systolic blood pressure (mm Hg)					
Men	127 ± 16	128 ± 17	128 ± 17	0.743	0.441
Women	122 ± 18	124 ± 20	124 ± 20	0.391	0.230
Diastolic blood pressure (mm Hg)					
Men	82 ± 10	81 ± 10	82 ± 10	0.414	0.542
Women	76 ± 10 <sup>6</sup>	77 ± 10	79 ± 10 <sup>7</sup>	0.022	0.010
Nondrinkers [ <i>n</i> (%)]					
Men	20 (16.0)	129 (18.7)	48 (24.2)	0.127	0.135
Women	46 (27.1)	241 (32.7)	55 (24.4)	0.087	0.363
Current smokers [ <i>n</i> (%)]					
Men	24 (19.2)	127 (18.4)	38 (19.2)	0.955	0.677
Women	30 (17.6)	142 (19.3)	48 (21.1)	0.677	0.378

<sup>1</sup>For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.

<sup>2</sup>By ANOVA or chi-square test for global comparison of means between *APOE* allele types.

<sup>3</sup>For linear trends between categories by ANOVA.

<sup>4</sup> $\bar{x} \pm \text{SD}$ .

<sup>5</sup>Significantly different from *E3*, *P* < 0.05 (Bonferroni adjustment for multiple comparisons).

<sup>6</sup>Significantly different from *E4*, *P* < 0.05 (Bonferroni adjustment for multiple comparisons).

<sup>7</sup>Significantly different from *E2*, *P* < 0.05 (Bonferroni adjustment for multiple comparisons).

<sup>8</sup>1 MJ = 239.23 kcal.

drinkers, significant differences in LDL cholesterol (*P* < 0.001) were observed between *APOE* allele types. Subjects with the *E2* allele had the lowest concentrations and subjects with the *E4* allele had the highest concentrations. We observed that LDL-cholesterol concentrations in drinkers with the *E2* allele were

significantly lower than those in nondrinkers with the *E2* allele (2.84 compared with 3.28 mmol/L). Conversely, mean LDL cholesterol was higher in drinkers than in nondrinkers with the *E4* allele (3.54 compared with 3.09 mmol/L; *P* < 0.001). In subjects with the *E3* allele, mean LDL-cholesterol concentrations did not

**TABLE 3**  
Pearson correlation coefficients between intakes of dietary fat, energy, and alcohol by sex and by *APOE* allele type<sup>1</sup>

Dietary intake	Men (n = 1014)				Women (n = 1133)			
	Total fat	Saturated fat	Cholesterol	Energy intake	Total fat	Saturated fat	Cholesterol	Energy intake
Saturated fat (g/d)	0.947 <sup>2</sup>				0.947 <sup>2</sup>			
<i>E2</i>	0.905 <sup>2</sup>				0.958 <sup>2</sup>			
<i>E3</i>	0.944 <sup>2</sup>				0.949 <sup>2</sup>			
<i>E4</i>	0.941 <sup>2</sup>				0.943 <sup>2</sup>			
Cholesterol (mg/d)	0.718 <sup>2</sup>	0.733 <sup>2</sup>			0.738 <sup>2</sup>	0.741 <sup>2</sup>		
<i>E2</i>	0.804 <sup>2</sup>	0.830 <sup>2</sup>			0.721 <sup>2</sup>	0.715 <sup>2</sup>		
<i>E3</i>	0.702 <sup>2</sup>	0.723 <sup>2</sup>			0.752 <sup>2</sup>	0.755 <sup>2</sup>		
<i>E4</i>	0.712 <sup>2</sup>	0.735 <sup>2</sup>			0.780 <sup>2</sup>	0.766 <sup>2</sup>		
Energy (MJ/d)	0.846 <sup>2</sup>	0.784 <sup>2</sup>	0.628 <sup>2</sup>		0.838 <sup>2</sup>	0.770 <sup>2</sup>	0.648 <sup>2</sup>	
<i>E2</i>	0.845 <sup>2</sup>	0.780 <sup>2</sup>	0.688 <sup>2</sup>		0.843 <sup>2</sup>	0.783 <sup>2</sup>	0.703 <sup>2</sup>	
<i>E3</i>	0.847 <sup>2</sup>	0.784 <sup>2</sup>	0.607 <sup>2</sup>		0.837 <sup>2</sup>	0.766 <sup>2</sup>	0.643 <sup>2</sup>	
<i>E4</i>	0.872 <sup>2</sup>	0.815 <sup>2</sup>	0.638 <sup>2</sup>		0.864 <sup>2</sup>	0.806 <sup>2</sup>	0.696 <sup>2</sup>	
Alcohol (g/d)	-0.062 <sup>3</sup>	-0.055	-0.019	0.102 <sup>2</sup>	0.004	-0.014	0.012	0.082 <sup>2</sup>
<i>E2</i>	0.111	0.097	0.103	0.268 <sup>2</sup>	0.007	-0.003	0.038	0.083
<i>E3</i>	-0.038	-0.025	0.003	0.152 <sup>2</sup>	0.014	0.004	0.006	0.122 <sup>2</sup>
<i>E4</i>	-0.094	-0.090	-0.028	0.085	-0.001	-0.009	-0.038	-0.033

<sup>1</sup>For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.

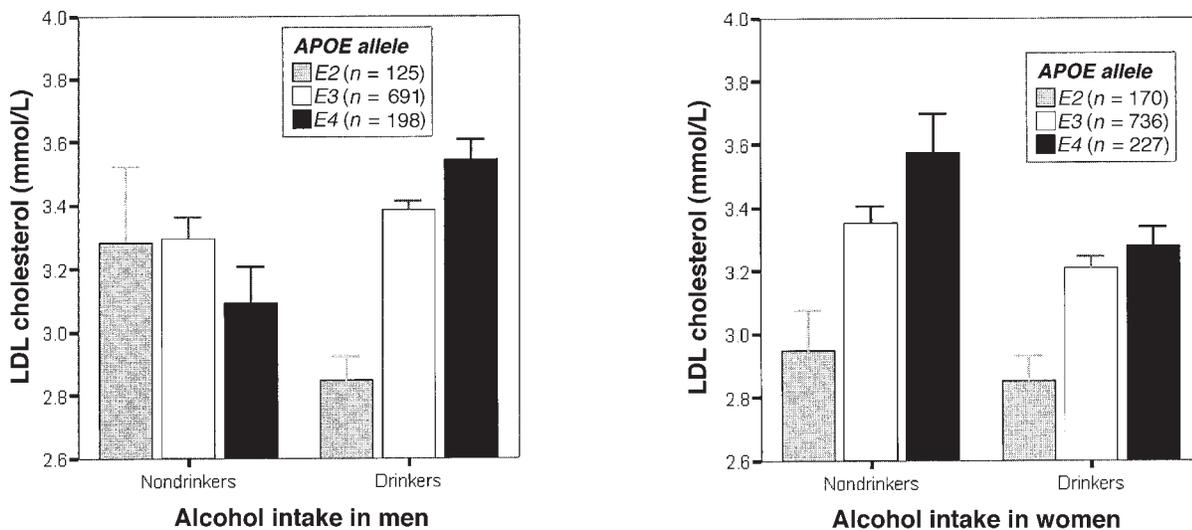
<sup>2</sup> $P < 0.01$ .

<sup>3</sup> $P < 0.05$ .

differ significantly by alcohol intake status (3.29 mmol/L in nondrinkers and 3.38 mmol/L in drinkers). In women, the expected effect of *APOE* alleles on LDL-cholesterol concentrations occurred in both drinkers ( $P < 0.001$ ) and nondrinkers ( $P < 0.001$ ). Similar results were obtained when mean LDL-cholesterol concentrations were stratified by age group (< and  $\geq 47$  y).

The results of regression models of the association between *APOE* alleles and alcohol intake, after control for possible confounding factors, are summarized for men and women separately in **Table 4**. We observed a highly significant interaction ( $P = 0.001$ ) between alcohol intake and the effect of *APOE* allele

status on LDL-cholesterol concentrations in men. In women, this interaction term was not significant. The results obtained with these models confirm the interaction between *APOE* allele group and alcohol intake shown in Figure 1. The interaction between alcohol intake and *APOE* allele status remained significant in men after age, BMI, saturated fat intake, energy intake, and tobacco smoking were controlled for. The regression coefficients for the interaction terms indicated that the LDL-cholesterol-lowering effect observed in carriers of the *E2* allele ( $-0.532$  mmol/L;  $P < 0.001$ ; Table 4) was absent in male nondrinkers (0.538 mmol/L;  $P < 0.01$ ). Likewise, the usual elevating effect observed in carriers



**FIGURE 1.** Mean ( $\pm$ SEM) plasma LDL-cholesterol concentrations by *APOE* genotype and alcohol intake status in men and women. ANOVA: in men, NS for nondrinkers and  $P < 0.001$  for drinkers; in women,  $P < 0.001$  for both nondrinkers and drinkers. There was a significant interaction ( $P < 0.01$ ) between alcohol intake and the effect of *APOE* allele type on LDL-cholesterol concentrations in men, but not in women. For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.

**TABLE 4**Effect of *APOE* allele type, alcohol intake, saturated fat intake, and *APOE*-alcohol interaction on plasma LDL cholesterol: multiple linear regression analysis by sex<sup>1</sup>

	Men (n = 1014)			Women (n = 1133)		
	$\beta \pm SE$	95% CI	P	$\beta \pm SE$	95% CI	P
<i>APOE</i> allele <sup>2</sup>			0.036			<0.001
<i>E3</i>	Reference			Reference		
<i>E2</i>	-0.532 ± 0.083	(-0.695, -0.369)	<0.001	-0.370 ± 0.080	(-0.527, -0.213)	<0.001
<i>E4</i>	0.157 ± 0.070	(0.020, 0.294)	0.028	0.056 ± 0.070	(-0.081, 0.193)	0.436
Alcohol intake			0.161			0.104
Consumption	Reference			Reference		
No consumption	-0.107 ± 0.075	(-0.254, 0.040)	0.161	0.066 ± 0.065	(-0.061, 0.193)	0.104
Saturated fat intake			0.077			0.593
≥14%	Reference			Reference		
10–14%	-0.171 ± 0.078	(-0.324, -0.018)	0.027	-0.031 ± 0.083	(-0.194, 0.132)	0.713
≤10%	-0.159 ± 0.075	(-0.306, -0.012)	0.045	-0.072 ± 0.083	(-0.235, 0.091)	0.389
<i>APOE</i> -alcohol interaction			0.001			0.474
<i>E3</i> × alcohol consumption	Reference			Reference		
<i>E2</i> × no alcohol consumption	0.538 ± 0.204	(0.138, 0.938)	0.009	-0.036 ± 0.153	(-0.336, 0.264)	0.814
<i>E4</i> × no alcohol consumption	-0.339 ± 0.147	(-0.627, -0.051)	0.024	0.160 ± 0.140	(-0.114, 0.434)	0.257
R <sup>2</sup> of the model <sup>3</sup>	0.071		<0.001	0.135		<0.001

<sup>1</sup>The dependent variable was LDL cholesterol (mmol/L).<sup>2</sup>For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.<sup>3</sup>Models additionally adjusted by age, BMI, tobacco smoking, and energy intake.

of the *E4* allele on LDL cholesterol (0.157 mmol/L;  $P < 0.05$ ) was also absent in male nondrinkers (-0.339 mmol/L;  $P < 0.05$ ). The interaction described between alcohol and *APOE* allele type was present in all categories of saturated fat intake. The interaction term between *APOE* allele type and saturated fat intake was not significant when included in these models and it was removed in subsequent analyses.

For the whole male cohort, the average effect of the *E2* allele on LDL-cholesterol concentrations was -0.334 mmol/L, the average effect of the *E3* allele was 0.034 mmol/L, and the average effect of the *E4* allele was 0.098 mmol/L. In drinking men, the average effects of the *E2*, *E3*, and *E4* alleles were -0.450, 0.002, and 0.160 mmol/L, respectively. In nondrinking men, the average effects of the *E2*, *E3*, and *E4* alleles were -0.004, 0.112,

and -0.004 mmol/L, respectively. These results agree with the data in Table 4 for the men carrying the *E2* or *E4* allele.

Mean LDL-cholesterol concentrations in men and women by *APOE* allele type and stratified by alcohol intake (no, moderate, and high) are shown in Table 5. The effects of *APOE* allele type were not significantly different between moderate and heavy drinkers. In men, the interaction term between *APOE* allele type and alcohol intake remained significant ( $P = 0.004$ ). However, only comparisons of nondrinkers with moderate or heavy drinkers were significant and there were no significant differences between moderate and heavy drinkers. In women, the interaction term between *APOE* allele type and alcohol intake was not significant.

Partial regression coefficients between alcohol intake (square root of the value in g/d) and LDL-cholesterol concentrations are

**TABLE 5**Plasma LDL-cholesterol concentrations by *APOE* allele type, stratified by alcohol intake<sup>1</sup>

<i>APOE</i> allele type	n	Men				n	Women				
		All men (n = 1014)	No intake (n = 197)	Moderate intake (n = 602)	High intake (n = 215)		All women (n = 1133)	No intake (n = 342)	Moderate intake (n = 593)	High intake (n = 198)	
		mmol/L									
<i>E2</i>	125	2.92 ± 0.86 <sup>2,4</sup>	3.28 ± 1.06	2.88 ± 0.82 <sup>3,4</sup>	2.68 ± 0.67 <sup>3,4</sup>	170	2.88 ± 0.90 <sup>3,4</sup>	2.95 ± 0.86 <sup>3,4</sup>	2.90 ± 0.95 <sup>3,4</sup>	2.71 ± 0.82 <sup>3,4</sup>	
<i>E3</i>	691	3.37 ± 0.77 <sup>5</sup>	3.30 ± 0.79	3.40 ± 0.78 <sup>5</sup>	3.35 ± 0.71 <sup>5</sup>	736	3.25 ± 0.85 <sup>5</sup>	3.35 ± 0.86 <sup>5</sup>	3.22 ± 0.83 <sup>5</sup>	3.18 ± 0.88 <sup>5</sup>	
<i>E4</i>	198	3.43 ± 0.83 <sup>5</sup>	3.09 ± 0.80	3.56 ± 0.77 <sup>5</sup>	3.49 ± 0.92 <sup>5</sup>	227	3.35 ± 0.82 <sup>5</sup>	3.57 ± 0.91 <sup>5</sup>	3.23 ± 0.76 <sup>5</sup>	3.40 ± 0.84 <sup>5</sup>	
All allele types	1014	3.33 ± 0.80	3.21 ± 0.83	3.35 ± 0.81	3.33 ± 0.78	1133	3.22 ± 0.86	3.33 ± 0.88	3.17 ± 0.84	3.16 ± 0.88	
P <sup>6</sup>		<0.001	0.341	<0.001	0.001	P <sup>6</sup>	<0.001	0.001	0.004	0.002	

<sup>1</sup>For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.<sup>2</sup> $\bar{x} \pm SD$ .<sup>3</sup>Significantly different from *E3*,  $P < 0.05$  (Bonferroni adjustment for multiple comparisons).<sup>4</sup>Significantly different from *E4*,  $P < 0.05$  (Bonferroni adjustment for multiple comparisons).<sup>5</sup>Significantly different from *E2*,  $P < 0.05$  (Bonferroni adjustment for multiple comparisons).<sup>6</sup>ANOVA for global comparison of means between allele types.

**TABLE 6**  
Association between alcohol intake and plasma LDL-cholesterol concentrations by *APOE* allele type<sup>1</sup>

	<i>n</i>	$\beta^2$	SE	<i>r</i> <sup>3</sup>	<i>P</i>
Men					
All men	1014	0.018	0.009	0.057	0.049
<i>E2</i>	125	-0.104	0.033	-0.279	0.002
<i>E3</i>	691	0.013	0.012	0.042	0.292
<i>E4</i>	198	0.064	0.024	0.198	0.008
Women					
All women	1133	-0.012	0.012	-0.026	0.299
<i>E2</i>	170	-0.012	0.034	-0.026	0.732
<i>E3</i>	736	-0.026	0.017	-0.056	0.122
<i>E4</i>	227	-0.013	0.027	-0.031	0.631

<sup>1</sup>The dependent variable was LDL cholesterol (mmol/L). For analytical purposes, genotypes *E2/E2* and *E2/E3* were grouped as *E2*, genotypes *E3/E4* and *E4/E4* as *E4*, and genotype *E3/E3* as *E3*. Subjects with the *E2/E4* genotype were excluded from the analysis.

<sup>2</sup>Partial regression coefficient for alcohol intake (square-root of g/d) adjusted for age, BMI, fat and energy intakes, and tobacco smoking.

<sup>3</sup>Partial correlation coefficient adjusted for age, BMI, fat and energy intakes, and tobacco smoking.

shown **Table 6**. In men carrying the *E2* allele, a negative and significant association ( $r = -0.279$ ,  $\beta = -0.104$ ,  $P = 0.002$ ) was found between alcohol intake and LDL-cholesterol concentrations. In men carrying the *E4* allele, the association between alcohol and LDL-cholesterol concentrations was positive and significant ( $r = 0.198$ ,  $\beta = 0.064$ ,  $P = 0.008$ ). The statistical associations were not significant in either men or women with the *E3* allele. When nondrinkers were excluded from the previous analysis (data not shown), only the negative association of alcohol intake and LDL-cholesterol concentrations observed for men with the *E2* allele remained significant ( $r = -0.214$ ,  $\beta = -0.082$ ,  $P < 0.05$ ).

## DISCUSSION

This study showed a significant interaction between alcohol intake and *APOE* allele type in men and supported the concept that the effects of *APOE* genetic variability on plasma lipid concentrations are dependent on environmental factors. The effects of *APOE* polymorphism on LDL-cholesterol concentrations (1–3) were not observed in nondrinking men. In this group, the expected lowering effect of the *E2* allele was absent and the elevating effect of the *E4* allele was reversed.

Previous studies reported significant interactions between *APOE* allele type and plasma lipid responses to both dietary and pharmacologic therapies (39). However, most of these studies did not investigate the effects of the interactions between alcohol intake and *APOE* allele type on lipid concentrations, and the diet studies focused on fat and cholesterol intakes rather than on alcohol intake. Although the results of these studies are controversial (40), it has been hypothesized that the contribution of the *E4* allele to elevated LDL-cholesterol concentrations is higher in the presence of an atherogenic diet (39). Moreover, several authors suggest that a low-fat diet can suppress the deleterious effects of the *E4* allele on plasma lipids (7, 41–43). Along these lines, a study carried out in a Native American rural population following a traditional lifestyle reported no significant differences in LDL-cholesterol concentrations between subjects with the *E3/E3* and *E3/E4* genotypes (7). Kamboh et al (41) reported

similar results in Mayan Indians living in Mexico. Considering that Native American populations living their traditional lifestyle do not consume large amounts of alcohol, our reported interaction with alcohol intake, in addition to the dietary fat hypothesis, could explain the results reported in those studies. This gene-environment interaction hypothesis could also be applied to studies reported by Deiana et al (42) and Sandholzer et al (43). However, these studies did not present data on diet and alcohol intake and the data were not stratified by sex.

The results of the present study support the importance of considering alcohol intake and sex when examining genotype-phenotype associations. In the present study, the inclusion of a sex interaction term in the multivariate model, stratification by alcohol intake and genotype, and the additional control for confounders such as dietary fat allowed the identification of an interaction between *APOE* allele type and alcohol intake. In studies in which these factors were not considered, the alcohol interaction effects may have been masked and therefore ignored.

Another subject of interest in studying gene-environment interactions is the influence of age (43). The *APOE* allelic effect may differ in the elderly (5, 6, 44, 45). This possible effect was assessed in male twins followed longitudinally for 15 y (5). At exam 1 (mean age: 48 y), total and LDL cholesterol were lower in the *E3* group than in the *E4* group, but at exam 3 (mean age: 63 y), total and LDL-cholesterol concentrations were higher in the *E3* group than in the *E4* group. Aging could also explain the lack of association between *E4* allele type and LDL-cholesterol concentrations reported in a multiethnic elderly population (6). The reasons for these age-related differences are unclear. Other studies showed that dietary fat and alcohol intakes are lower in the elderly (46–49). According to our data, the *APOE* allelic effects observed in the general population should not be present in population groups consuming little or no alcohol.

These effects may also apply to children. Although some studies (50, 51) showed an *APOE* allelic effect on LDL cholesterol in children, others did not (52, 53). Assuming that these children did not drink alcoholic beverages, these results are consistent with those observed in the nondrinking men in our study.

Although this is the first description of an *APOE*-alcohol interaction affecting lipid concentrations, Kauma et al (54) reported a male-specific interaction between *APOE* alleles and alcohol intake in a study to determine the effects of this interaction on blood pressure. The influence of sex differences on the effects of alcohol on plasma lipids have rarely been studied (55), possibly because of the added complexity introduced by hormonally induced changes in lipids during menopause (56), during hormonal therapy in women (57), and throughout the menstrual cycle (58). Estrogens may be crucial in determining the effect of alcohol and the possible interaction between alcohol and *APOE* genotype. An interaction with estrogens may explain the reported gene-sex interactions affecting several loci and environmental factors (39, 59). However, the lack of information on hormone concentrations in the population we studied prevented us from verifying this interaction.

In interpreting our findings, we should consider several aspects. First, this is a cross-sectional analysis and we did not determine the temporal sequence of the observed results. Second, alcohol intake was self-reported and may have been subject to bias. The nondifferential response error in the Framingham Study was estimated to be low (60). In addition, alcohol intake was used mainly as a 2-category variable (intake or no intake),

which eliminates bias in the estimation of the amount consumed. Factors affecting this dichotomized classification may have been more important in our study. However, the denial of alcohol use by drinkers was likely low, considering the excellent rapport between the participants and the investigators in the Framingham Offspring Study (25). The possibility that nondrinkers declare some alcohol consumption is remote (61). Finally, the limitation pointed out by Shaper (62), arguing that a group of supposed nondrinkers usually consists of subjects who have reduced their alcohol intake because of preexisting disease, was minimized in the present study because subjects with CHD or who were taking lipid-lowering medication were excluded. Another issue relates to the possible confounding of fat intake on the effect of alcohol. In populations whose intakes of fat and cholesterol vary with alcohol intake, diet may distort the relation between alcohol intake and CHD in general or that between alcohol intake and LDL cholesterol in particular (63). Thus, because fat intake differs among alcohol drinkers (64), the association between alcohol intake and CHD is more accurate if dietary habits are controlled for. In the present study, the initial effect described for alcohol remained after fat and energy intakes were controlled for in the multivariate models. In addition, BMI and tobacco smoking were controlled for in our analyses because alcohol intake was shown previously to be directly associated with tobacco smoking and several studies found a lower BMI in drinkers than in nondrinkers (65, 66). Because other potential confounding factors, such as physical activity, may still vary among subjects with different alcohol intakes, we cannot exclude the possibility that this finding may represent a spurious association. All of these factors need to be examined.

The biochemical mechanism underlying the reported interaction is unknown. Considering the numerous metabolic pathways potentially influenced by ethanol (67), several hypotheses are possible, including effects on endogenous hepatic and intestinal cholesterol synthesis, increased rates of intestinal cholesterol absorption, activation of lipoprotein lipase, inhibition of cholesteryl ester transfer protein, LDL modification, and consequently a change in the LDL-receptor binding affinity (68–71). It is known that the *E2* allele has a low affinity for binding to LDL receptors (45). In addition, LDL particles in alcohol drinkers are smaller and probably acetylated, which also reduces their affinity for LDL receptors (70, 72). In the absence of alcohol, men with the *E2* allele may have LDL-cholesterol concentrations similar to those of individuals with the *E3* allele, but in the presence of alcohol the lower affinity of the *E2* allele to the receptors may be magnified, resulting in an increase in LDL receptors and a decrease in LDL-cholesterol concentrations. In subjects with the *E4* allele, VLDLs are apo E enriched and these particles may be taken up more readily by the liver, thus decreasing the expression of LDL receptors and increasing plasma LDL-cholesterol concentrations. Alcohol induces an increase in postprandial lipemia and in triacylglycerol-rich lipoprotein remnants, resulting in greater cholesterol uptake by hepatic receptors, which will down-regulate LDL receptors even farther and enhance the cholesterol-raising effect of *E4* (73, 74).

Although cause and effect were not shown in this study, our findings support the hypothesis that the effects of *APOE* allele type on plasma lipids and CHD risk are modulated by environmental factors. The interaction effect of alcohol and *APOE* alleles on LDL-cholesterol concentrations in men was consistent and independent of saturated fat and energy intakes, smoking, or

BMI. However, we did not detect a similar effect in women, suggesting a gene-sex interaction. Our data also suggest that part of the variability in the effects of alcohol drinking on LDL-cholesterol concentrations may have been due to the sex distribution and genetic makeup of the sample studied. 

## REFERENCES

1. Eichner JE, Kuller LH, Ferrell RE, Meilahn EN, Kamboh MI. Phenotypic effects of apolipoprotein structural variation on lipid profiles. III. Contribution of apolipoprotein E phenotype to prediction of total cholesterol, apolipoprotein B, and low density lipoprotein cholesterol in the healthy women study. *Arteriosclerosis* 1990;10:379–85.
2. Boerwinkle E, Utermann G. Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. *Am J Hum Genet* 1988;42:104–12.
3. Wilson PWF, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease: The Framingham Offspring Study. *JAMA* 1994;272:1666–71.
4. Braeckman L, De Bacquer D, Rosseneu M, De Backer G. Apolipoprotein E polymorphism in middle-aged Belgian men: phenotype distribution and relation to serum lipids and lipoproteins. *Atherosclerosis* 1996;120:67–73.
5. Jarvik GP, Austin MA, Fabsitz RR, et al. Genetic influences on age-related change in total cholesterol, low density lipoprotein cholesterol, and triglyceride levels: longitudinal apolipoprotein E genotype effects. *Genet Epidemiol* 1994;11:375–84.
6. Pablos-Mendez A, Mayeux R, Ngai C, Shea S, Berglund L. Association of apo E polymorphism with plasma lipid levels in a multiethnic elderly population. *Arterioscler Thromb Vasc Biol* 1997;17:3534–41.
7. 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* 1999;142:409–14.
8. Lopez-Miranda J, Ordovas JM, Mata P, et al. Effect of apolipoprotein E phenotype on diet-induced lowering of plasma low density lipoprotein cholesterol. *J Lipid Res* 1994;35:1965–75.
9. Lehtimäki T, Frankberg-Lakkala H, Solakivi T, et al. The effect of short-term fasting, apolipoprotein E gene polymorphism, and sex on plasma lipids. *Am J Clin Nutr* 1997;66:599–605.
10. Kiechl S, Willeit J, Rungger G, Egger G, Oberhollenzer F, Bonora E. Alcohol consumption and atherosclerosis: what is the relation? Prospective results from the Bruneck Study. *Stroke* 1998;29:900–7.
11. McElduff P, Dobson AJ. How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *BMJ* 1997;314:1159–64.
12. Gronbaek M, Deis A, Becker U, et al. Alcohol and mortality: is there a U-shaped relation in elderly people? *Age Ageing* 1998;27:739–44.
13. Renaud SC, Gueguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from eastern France. *Epidemiology* 1998;9:184–8.
14. Keil U, Chambless LE, Döring A, Filipiak B, Stieber J. The relation of alcohol intake to coronary heart disease and all-cause mortality in a beer-drinking population. *Epidemiology* 1997;8:150–6.
15. Rimm EB, Klatsky A, Grobbee D, Stampfer MJ. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. *BMJ* 1996;312:731–6.
16. Glueck CJ, Hogg E, Allen C, Gartside PS. Effects of alcohol ingestion on lipids and lipoproteins in normal men: isocaloric metabolic studies. *Am J Clin Nutr* 1980;33:2287–93.
17. Gaziano JM, Buring JE, Breslow JL, et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N Engl J Med* 1993;329:1829–34.
18. Rimm EB, Williams P, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ* 1999;319:1523–8.

19. Vasisht S, Pant MC, Srivastava LM. Effect of alcohol on serum lipids & lipoproteins in male drinkers. *Indian J Med Res* 1992;96:333-7.
20. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. *Circulation* 1992;85:910-5.
21. Nakanishi N, Nakamura K, Ichikawa S, Suzuki K, Tatara K. Relationship between lifestyle and serum lipid and lipoprotein levels in middle-aged Japanese men. *Eur J Epidemiol* 1999;15:341-8.
22. McConnell MV, Vavouranakis I, Wu LL, Vaughan DE, Ridker PM. Effects of a single, daily alcoholic beverage on lipid and hemostatic markers of cardiovascular risk. *Am J Cardiol* 1997;80:1226-8.
23. Rakic V, Puddey IB, Dimmitt SB, Burke V, Beilin LJ. A controlled trial of the effects of pattern of alcohol intake on serum lipid levels in regular drinkers. *Atherosclerosis* 1998;137:243-52.
24. Hein HO, Suadicani P, Gyntelberg F. Alcohol consumption, serum low density lipoprotein cholesterol concentration, and risk of ischaemic heart disease: six year follow up in the Copenhagen male study. *BMJ* 1996;312:736-41.
25. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study. Design and preliminary data. *Prev Med* 1975;4:518-25.
26. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol* 1979;110:281-90.
27. Dawber TR, Meadors GF, Moore R. Epidemiological approaches to heart disease: The Framingham Study. *Am J Public Health* 1951;41:279-86.
28. Cupples LA, Gagnon DR, Kannel WB. Long- and short-term risk of sudden coronary death. *Circulation* 1992;85:111-8.
29. Warnick R, Benderson J, Albers JJ. Dextran sulfate-Mg precipitation procedure for quantitation of high density lipoprotein cholesterol. *Clin Chem* 1982;28:1379-88.
30. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
31. McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma and apolipoprotein fractions. *Clin Chim Acta* 1987;166:1-9.
32. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1989;16:1215.
33. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI*. *J Lipid Res* 1990;31:545-8.
34. Willett WC, Sampson LA, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51-65.
35. Tucker KL, Rich S, Rosenberg I, et al. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. *Am J Clin Nutr* 2000;71:514-22.
36. Pearson TA. Alcohol and heart disease. *Circulation* 1996;94:3023-5.
37. Cannings C, Edwards AW. Expected genotypic frequencies in a small sample: deviation from Hardy-Weinberg equilibrium. *Am J Hum Genet* 1969;21:245-47.
38. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am J Clin Genet* 1985;37:268-85.
39. Ordovas JM. The genetics of serum lipid responsiveness to dietary interventions. *Proc Nutr Soc* 1999;58:171-87.
40. Lefevre M, Ginsberg HN, Kris-Etherton PM, et al. ApoE genotype does not predict lipid response to changes in dietary saturated fatty acids in a heterogeneous normolipidemic population. The DELTA Research Group. Dietary effects on lipoproteins and thrombogenic activity. *Arterioscler Thromb Vasc Biol* 1997;17:2914-23.
41. Kamboh MI, Weiss KM, Ferrell RE. Genetic studies of human apolipoproteins. XVI. *APOE* polymorphism and cholesterol levels in the Mayans of the Yucatan Peninsula, Mexico. *Clin Genet* 1991;39:26-32.
42. Deiana L, Pes GM, Carru C, et al. Lack of influence of apolipoprotein E4 on lipoprotein levels in the island population of Sardinia. *Eur J Clin Invest* 1998;28:290-4.
43. Sandholzer C, Delport R, Vermaak H, Utermann G. High frequency of the apo epsilon 4 allele in Khoi San from South Africa. *Hum Genet* 1995;95:46-8.
44. 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* 1996;143:463-78.
45. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 1988;8:1-21.
46. Whichelow MJ, Prevost AT. Dietary patterns and their associations with demographic, lifestyle and health variables in a random sample of British adults. *Br J Nutr* 1996;76:17-30.
47. Posner BM, Cupples LA, Miller DR, Cobb JL, Lutz KJ, D'Agostino RB. Diet, menopause, and serum cholesterol levels in women: The Framingham Study. *Am Heart J* 1993;125:483-9.
48. Shaper AG, Wannamethee SG. The J-shaped curve and changes in drinking habit. *Novartis Found Symp* 1998;216:173-88.
49. Greenfield TK, Rogers JD. Who drinks most of the alcohol in the US? The policy implications. *J Stud Alcohol* 1999;60:78-89.
50. Okada T, Sato Y, Iwata F, Hara M, Kim H, Harada K. Relationship of apolipoprotein E phenotypes to serum lipid and lipoprotein levels in Japanese schoolchildren. *Acta Paediatr* 1998;87:460-1.
51. Lehtimäki T, Porkka K, Viikari J, Ehnholm C, Åkerblom HK, Nikkari T. Apolipoprotein E phenotypes and serum lipids in newborns and 3-year-old children: the Cardiovascular Risk in Young Finns Study. *Pediatrics* 1994;94:489-93.
52. Dixon LB, Shannon BM, Tershakovec AM, Bennett MJ, Coates PM, Cortner JA. Effects of family history of heart disease, apolipoprotein E phenotype, and lipoprotein(a) on the response of children's plasma lipids to change in dietary lipids. *Am J Clin Nutr* 1997;66:1207-17.
53. Bercedo A, Gonzalez-Lamuno D, Muñoz O, et al. Association between lipid profile and Apo E genotype in Spanish children (8-15 years old). *Ann Esp Pediatr* 1998;42:120-4.
54. Kauma H, Savolainen MJ, Rantala AO, et al. Apolipoprotein E phenotype determines the effect of alcohol on blood pressure in middle-aged men. *Am J Hypertens* 1998;11:1334-43.
55. Lin RC, Miller BA, Kelly TJ. Concentrations of apolipoprotein AI, AII, and E in plasma and lipoprotein fractions of alcoholic patients: gender differences in the effects of alcohol. *Hepatology* 1995;21: 942-9.
56. Higuchi M, Iwaoka K, Ishii K, et al. Plasma lipid and lipoprotein profiles in pre- and post-menopausal middle-aged runners. *Clin Physiol* 1990;10:69-76.
57. Garry PJ, Baumgartner RN, Brodie SG, et al. Estrogen replacement therapy, serum lipids, and polymorphism of the apolipoprotein E gene. *Clin Chem* 1999;45:1214-23.
58. Jones DY, Judd JT, Taylor PR, Campbell WS, Nair PP. Menstrual cycle effect on plasma lipids. *Metabolism* 1988;37:1-2.
59. Hegele RA, Evans AJ, Tu L, Ip G, Brunt JH, Connelly PW. A gene-gender interaction affecting plasma lipoproteins in a genetic isolate. *Arterioscler Thromb* 1994;14:671-8.
60. Posner BM, Martin-Munley SS, Smigelski C, et al. Comparison of the techniques for estimating nutrient intake: The Framingham Study. *Epidemiology* 1992;3:171-7.
61. Romelsjö A, Leifman H, Nystrom SA. A comparative study of two methods for the measurement of alcohol consumption in the general population. *Int J Epidemiol* 1995;24:929-36.
62. Shaper AG. Alcohol and mortality: a review of prospective studies. *Br J Addict* 1990;85:837-47.
63. Colditz GA, Giovannucci E, Rimm EB, et al. Alcohol intake in relation to diet and obesity in women and men. *Am J Clin Nutr* 1991;54:49-55.
64. Mannisto S, Uusitalo K, Roos E, Fogelholm M, Pietinen P. Alcohol beverage drinking, diet and body mass index in a cross-sectional survey. *Eur J Clin Nutr* 1997;51:326-32.

65. Wannamethee G, Shaper AG. Blood lipids: the relationship with alcohol intake, smoking, and body weight. *J Epidemiol Community Health* 1992;46:197-202.
66. Bottoni A, Cannella C, Del Balzo V. Lifestyle and dietary differences in smokers and non-smokers from an Italian employee population. *Public Health* 1997;111:161-4.
67. Frohlich JJ. Effects of alcohol on plasma lipoprotein metabolism. *Clin Chim Acta* 1996;246:39-49.
68. Liinamaa MJ, Hannuksela ML, Kesaniemi YA, Savolainen MJ. Altered transfer of cholesteryl esters and phospholipids in plasma from alcohol abusers. *Arterioscler Thromb Vasc Biol* 1997;17:2940-7.
69. Simon JA, Fong J, Bernert JT Jr, Browner NS. Relation of smoking and alcohol consumption to serum fatty acids. *Am J Epidemiol* 1996;144:325-34.
70. Hirano K, Yamashita S, Sakai N, et al. Low-density lipoproteins in hyperalphalipoproteinemic heavy alcohol drinkers have reduced affinity for the low-density lipoprotein receptor. *Clin Biochem* 1992;25:357-62.
71. Nishiwaki M, Ishikawa T, Ito T, et al. Effects of alcohol on lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein, and lecithin:cholesterol acyltransferase in high-density lipoprotein cholesterol elevation. *Atherosclerosis* 1994;111:99-109.
72. Ayaori M. Beneficial effects of alcohol withdrawal on LDL particle size distribution and oxidative susceptibility in subjects with alcohol-induced hypertriglyceridemia. *Arterioscler Thromb Vasc Biol* 1997;17:2540-7.
73. Hendriks HFJ, Veenstra J, Van Tol A, Groener JE, Schaafsma G. Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition. *Alcohol* 1998;33:403-10.
74. Van Tol A, Van der Gaag MS, Scheek LM, Van Gent T, Hendriks HFJ. Changes in postprandial lipoproteins of low and high density caused by moderate alcohol consumption with dinner. *Atherosclerosis* 1998;141:S101-3.