Polyunsaturated fatty acids modulate the effects of the *APOA1* G-A polymorphism on HDL-cholesterol concentrations in a sex-specific manner: the Framingham Study[1–3]

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ABSTRACT

Background: A common G-to-A substitution in the promoter area (-75 base pairs) of the apolipoprotein A-I gene (*APOA1*) has been described. The *A* allele was shown to be associated with higher HDL-cholesterol concentrations in some studies but not in others.
Objective: We examined whether dietary fat modulates the association between this polymorphism and HDL-cholesterol concentrations.
Design: We studied a population-based sample of 755 men and 822 women from the Framingham Offspring Study.
Results: The frequency of the *A* allele was 0.165. No significant differences were observed between *G/G* subjects and carriers of the *A* allele for any lipid variables. In multivariate linear regression models, HDL-cholesterol concentrations in women were associated with a significant interaction between polyunsaturated fatty acid (PUFA) intake as a continuous variable and *APOA1* genotype ($P = 0.005$). By using 3 categories of PUFA intake, we found a significantly different effect of *APOA1* genotype across PUFA categories in women. When PUFA intake was <4% of energy, *G/G* subjects had ≈14% higher HDL-cholesterol concentrations than did carriers of the *A* allele ($P < 0.05$). Conversely, when PUFA intake was >8%, HDL-cholesterol concentrations in carriers of the *A* allele were 13% higher than those of *G/G* subjects ($P < 0.05$). No significant allelic difference was observed for subjects in the range of PUFA intake of 4–8% of energy. These interactions were not significant in men.
Conclusions: We found a significant gene-diet interaction associated with the *APOA1* G-A polymorphism. In women carriers of the *A* allele, higher PUFA intakes were associated with higher HDL-cholesterol concentrations, whereas the opposite effect was observed in *G/G* women. *Am J Clin Nutr* 2002;75:38–46.

INTRODUCTION

The recently revised dietary guidelines from the American Heart Association reflect an awareness that human genetic and metabolic heterogeneity could be used to adjust population-based nutritional guidelines so that they are optimal for individuals (1). Likewise, the summary of a conference on preventive nutrition sponsored by the American Heart Association emphasizes that we must identify the roles of specific polymorphic forms of genes that influence individual susceptibility to specific dietary factors (2). Along these lines, several loci have already been investigated for potential gene-diet interactions in the response to diet therapy (3). One of these is the *APOA1* locus that maps to the long arm of chromosome 11 (4). Its gene product, apolipoprotein (apo) A-I, plays a central role in lipid metabolism and coronary heart disease (CHD) risk (5–7).

A common G-to-A transition located 75 base pairs (bp) upstream from the transcription start site of the *APOA1* gene has been studied extensively. Initial reports showed that individuals carrying the *A* allele had higher concentrations of apo A-I and HDL cholesterol than did individuals with the *G/G* wild type (8, 9). Subsequently, studies that examined this association reported contradictory results. Although some were in partial agreement with the initial findings (10–17), others did not detect any significant association (18–23), and even an opposite association has been reported (24). A meta-analysis that included some of these studies showed that the rare *A* allele may be associated with mildly increased (by ≈0.05 g/L) apo A-I concentrations (25). It was suggested that the inconsistencies between studies could be the result of interactions with environmental factors that modulate the effect of this genetic polymorphism. Although some of these studies investigated the possible interaction with tobacco smoking (13–15, 17), none assessed the influence of long-term dietary habits in a large, population-based sample.

Dietary intervention studies including subjects with normal or slightly elevated cholesterol concentrations showed that the *A* allele is associated with increased LDL-cholesterol response to changes in dietary fat (26, 27). In one study (27), male and female subjects were first fed a diet high in saturated fatty acids

(SFAs), followed by a diet rich in monounsaturated fatty acids (MUFAs) and a diet rich in polyunsaturated fatty acids (PUFAs). The hypocholesterolemic effect associated with a high-PUFA diet was significantly greater in *G/A* women than in *G/G* women. In men, however, the *A* allele was not a predictor of response. In another study (26), young male subjects were fed a low-fat diet followed by a diet rich in MUFAs. After consumption of the high-MUFA diet, plasma LDL-cholesterol concentrations increased significantly in *G/A* subjects but not in *G/G* subjects. Although the dietary interventions and subject characteristics differed between these 2 studies, in both studies carriers of the *A* allele were more responsive to dietary changes than were *G/G* subjects (27). Conversely, no significant gene-diet interactions were observed in subjects who were heterozygotes for familial hypercholesterolemia (28) or in subjects participating in a dietary intervention study in North Karelia (10).

It has been established that dietary fatty acids, mainly PUFAs, can modulate gene expression (29). Considering that the G-A polymorphism discussed above is located in a GC-rich DNA region, it was proposed that the presence of A or G may differentially influence the efficiency of *APOA1* gene transcription. This hypothesis was tested in a series of in vitro experiments (30–34). However, these studies showed as much variability as did the population studies. Several reports found that the *A* allele was associated with lower transcriptional efficiency (30–32). Conversely, other investigators found increases in transcriptional efficiency associated with the *A* allele (34) or found no effect at all (33). Therefore, it was suggested that the observed effects could be a result of linkage disequilibrium with other regulatory sequences and that the combination of these elements may explain the contradictory results regarding *APOA1* gene expression (16, 33).

We designed the present study on the basis of the gene-environment interaction hypothesis put forward to reconcile the discrepancies associated with this common genetic variant. The goal of our study was to examine whether dietary fat modulates the association between the *APOA1* G-A polymorphism at −75 bp and HDL-cholesterol concentrations in a population-based sample of men and women in the Framingham Offspring Study.

SUBJECTS AND METHODS

Subjects and study design

The details of the design and methods of the Framingham Offspring Study are published elsewhere (35). Starting in 1971, 5124 subjects were enrolled (36). Blood samples for DNA analysis were collected between 1987 and 1991. Lipid phenotypes, DNA genotypes, and information on CHD risk factors and diet were available for 1362 men and 1572 women who attended the fifth examination visit, conducted between 1992 and 1995. Nearly all of the subjects were white. Data on smoking, height, and weight were obtained from these subjects as described previously (36, 37). Subjects with CHD, including myocardial infarction, angina pectoris, and coronary insufficiency, and those taking a lipid-lowering medication or receiving estrogen replacement therapy were excluded from this analysis. Subjects with plasma triacylglycerol concentrations >4.52 mmol/L were also excluded. Statistical analyses were performed for 755 men and 822 women who had complete data for all the variables examined. The mean age, for both men and women, was 55 y (range: 28–79 y). All procedures were approved by the institutional human investigation reviews boards.

Plasma lipid, lipoprotein, and apolipoprotein measurements

After subjects fasted for 12 h, venous blood samples were collected in tubes containing 0.1% EDTA. Plasma was separated from blood cells by centrifugation ($1600 \times g$ for 10 min at 4 °C) and was analyzed immediately for lipid content. Plasma total cholesterol, HDL cholesterol, and triacylglycerol concentrations were measured as described previously (38). HDL cholesterol was measured after precipitation of apo B–containing lipoproteins with dextran sulfate and magnesium sulfate. LDL-cholesterol concentrations were estimated by using the equation of Friedewald et al (39). Plasma concentrations of apo A-I and apo B were measured with a noncompetitive enzyme-linked immunosorbent assay by using affinity-purified polyclonal antibodies (40, 41). CVs for total cholesterol, HDL-cholesterol, and triacylglycerol measurements were all <5%.

DNA isolation and genotyping

Leukocyte DNA was extracted from 5–10 mL whole blood by using the method described by Miller et al (42). Amplification of a 432-bp region of the *APOA1* 5′ region was performed with the polymerase chain reaction in a DNA thermal cycler (PTC-100; MJ Research Inc, Watertown, MA) by using 250 ng genomic DNA and 0.2 μmol/L of each oligonucleotide primer (P1, 5′-AGGGACAGAGCTGATCCTTGAACTCTTAAG-3′; P2, 5′-TTAGGGGACACCTACCCGTCAGGAAGAGCA-3′) in a 50-μL volume. Each reaction mixture was heated at 95 °C for 5 min and followed by 30 cycles of amplification (95 °C for 1 min, 58 °C for 1.5 min, and 72 °C for 2 min). The polymerase chain reaction products were digested with 10 units of the restriction endonuclease enzyme *Msp*I (BRL, Rockville, MD) and the fragments were separated by using electrophoresis on a 3.5%-agarose gel. After electrophoresis, the gel was treated with ethidium bromide for 20 min and DNA fragments were visualized with ultraviolet illumination.

Dietary information

Dietary intake was estimated with the semiquantitative food-frequency questionnaire described by Rimm et al (43). This questionnaire includes 136 food items, with questions about intake of beer, wine, and spirits. Subjects are asked how often they consume each item per day, week, or month. Food-item intake frequencies are linked with nutrient data to estimate daily nutrient intakes.

Fat intake data were obtained in terms of absolute amounts (g/d). We then modeled the effect of fat in terms of nutrient density, ie, the ratio of energy from fat to total energy, expressed as a percentage. Intakes of total fat, SFAs, MUFAs, and PUFAs were calculated for each individual. These were included in the analyses as both continuous and categorical variables. To construct the categorical variables, intakes were classified into 2 groups according to the mean value of the population (ie, one group had intakes below the mean and one group had intakes above it). In addition, we defined 3 categories of PUFA intake (low, <4% of energy; middle, 4–8% of energy; and high, >8% of energy) on the basis of the frequency distribution and range of PUFA consumption in the population. PUFA intake ranged from 2.0% to 16.5% of total energy intake in men and from 1.2% to 13.7% of total energy intake in women.

Alcohol consumption was calculated in g/d on the basis of the individual's reported frequency of consumption of alcoholic beverages during the previous year. Subjects were also classified as either nondrinkers (those who did not report consumption

TABLE 1
Demographic, biochemical, dietary, and genotypic characteristics of the subjects[1]

	Men (n = 755)	Women (n = 822)
Age (y)	55.0 ± 9.4[2]	54.3 ± 9.5
BMI (kg/m^2)	28.2 ± 4.1	26.5 ± 5.4
Total cholesterol (mmol/L)	5.20 ± 0.91	5.37 ± 0.98
LDL cholesterol (mmol/L)	3.32 ± 0.82	3.22 ± 0.86
HDL cholesterol (mmol/L)	1.12 ± 0.29	1.46 ± 0.40
Triacylglycerol (mmol/L)	1.78 ± 1.43	1.48 ± 1.03
Apolipoprotein A-I (g/L)	1.42 ± 0.21	1.63 ± 0.26
Apolipoprotein B (g/L)	1.17 ± 0.58	1.09 ± 0.28
Total fat intake (g/d)	68.0 ± 27.8	56.5 ± 24.1
Saturated fat intake (g/d)	24.4 ± 10.9	20.2 ± 9.4
Monounsaturated fat intake (g/d)	26.2 ± 11.1	21.3 ± 9.4
Polyunsaturated fat intake (g/d)	12.8 ± 5.6	11.5 ± 5.3
Energy intake (kJ/d)	8448 ± 2749	7213 ± 2386
Alcohol intake (g/d)	15.9 ± 20.1	7.2 ± 11.8
Alcohol intake in drinkers (g/d)[3]	19.6 ± 20.4	10.1 ± 12.7
Cigarette smoking in smokers (no./d)[4]	23.7 ± 12.8	19.4 ± 10.4
APOA1 genotype [n (%)]		
G/G	530 (70.2)	581 (70.7)
G/A	197 (26.1)	214 (26.0)
A/A	28 (3.7)	27 (3.3)

[1] All variables except age and genotype distribution differed significantly by sex, P < 0.05.

[2] $\bar{x}$ ± SD.

[3] 80.0% of men and 73.3% of women were drinkers.

[4] 20.9% of men and 18.8% of women were smokers.

of alcohol) or as drinkers (those who reported drinking any amount of alcohol).

Statistical analyses

Triacylglycerol and apo B concentrations were log transformed and alcohol intake was square-root transformed to improve normality for statistical testing. Allele frequencies were estimated with the gene-counting method. Chi-square tests were conducted to examine whether the genotype frequencies were in Hardy-Weinberg equilibrium. To compare means between 2 independent groups, the Student's *t* test was used. For multiple comparisons of means, one-way analyses of variance were performed and P values for linear trends across categories were calculated by partitioning the between-groups sums of squares into trend components. Because of the marked sex differences in the variables of interest and the statistical significance of the interaction terms for sex in the regression models, statistical analyses were done separately for men and women. Pearson's product-moment correlation coefficients were calculated to describe unadjusted associations among continuous variables.

Multiple linear regression models with dummy variables for categorical terms were fitted to test the null hypotheses of no association between *APOA1* polymorphism and HDL-cholesterol or apo A-I concentrations (dependent variables) after considering the effects of several predictors (body mass index, age, sex, smoking, alcohol consumption, fat intake, energy intake, and *APOA1* polymorphism). Homogeneity of allelic effects according to sex or environmental factors was also tested by introducing the corresponding terms of interaction in a hierarchical way in the more parsimonious linear regression model. Regression coefficients and the proportion of variance attributable to each predictor were estimated from the models. For

HDL-cholesterol and apo A-I variables, 6 regression models were fitted separately for men and women. In the core model (model 1), no interaction terms with fat intakes (as continuous variables) were considered. In models 2–6, interaction terms between *APOA1* polymorphism (dichotomous) and intakes of total fat, SFAs, MUFAs, or PUFAs were tested. Additional multiple linear regression models with categorical variables for fat intake and for testing a gene-dosage effect for *APOA1* polymorphism were fitted as described in the Results section. Regression diagnostics such as analysis of residuals and colinearity tests were used to check the assumptions and to assess the accuracy of the computations. All reported P values were two-sided, and 95% CIs for estimated coefficients were calculated. The SAS statistical package (version 6.12; SAS Institute Inc, Cary, NC) was used for the analyses.

RESULTS

Subject characteristics

Anthropometric, biochemical, dietary, and genetic data from the subjects are shown in **Table 1**. No deviation from the Hardy-Weinberg equilibrium was observed (chi-square = 1.74; P > 0.05) for genotype distribution. The allele frequencies were 0.835 (95% CI: 0.822, 0.848) for the *G* allele and 0.165 (95% CI: 0.152, 0.178) for the *A* allele. There were statistically significant differences between men and women for all the variables examined, with the exceptions of age and genotype distribution.

Because of the small sample size of the *A/A* group (28 men and 27 women), subjects with *G/A* and *A/A* genotypes were combined and compared with the *G/G* homozygotes. Plasma lipid, lipoprotein, and apolipoprotein concentrations and dietary fat intakes by sex in *G/G* homozygotes and in carriers of the *A* allele are shown in **Table 2**. No significant differences between *G/G* homozygotes and carriers of the *A* allele were observed for age, body mass index, or any lipid, lipoprotein, or apolipoprotein variables. In women, mean HDL-cholesterol concentrations in the *G/G* and *G/A + A/A* groups were 1.46 ± 0.40 and 1.48 ± 0.41 mmol/L, respectively (NS). Likewise, male *G/G* homozygotes had HDL-cholesterol concentrations that were not significantly different from those of A-allele carriers (1.11 ± 0.29 and 1.12 ± 0.30 mmol/L, respectively; NS). Similar results were found for apo A-I concentrations. Mean dietary intakes of total fat, SFAs, MUFAs, and PUFAs did not differ significantly between the *G/A + A/A* and *G/G* groups.

Dietary fat intakes, *APOA1* alleles, and HDL-cholesterol concentrations

To test the hypothesis that dietary fat intake interacts with the *APOA1* G-A polymorphism in determining HDL-cholesterol concentrations, we controlled for possible confounding factors and then fitted several multivariate linear regression models (as described in the Methods) for men and women separately. The results for women are shown in **Table 3**. First, we fitted a core model (model 1) that included HDL-cholesterol concentration as the outcome variable and *APOA1* genotype (*G/G* as reference), alcohol consumption (drinkers as reference), tobacco smoking (nonsmokers as reference), age, body mass index, and total energy intake as predictor variables. In model 1, *APOA1* genotype adjusted for the other factors was not significantly related to

TABLE 2
Mean plasma lipid, lipoprotein, and apolipoprotein concentrations and dietary fat intakes by *APOA1* genotype and sex[1]

	APOA1 genotype	
	G/G	G/A + A/A
Age (y)		
Men	55.0 ± 9.4	55.02 ± 9.25
Women	54.3 ± 9.6	54.28 ± 9.46
BMI (kg/m²)		
Men	28.1 ± 4.1	28.28 ± 3.96
Women	26.5 ± 5.3	26.26 ± 5.28
Total cholesterol (mmol/L)		
Men	5.20 ± 0.94	5.21 ± 0.86
Women	5.35 ± 1.00	5.37 ± 0.90
LDL cholesterol (mmol/L)		
Men	3.32 ± 0.80	3.33 ± 0.79
Women	3.21 ± 0.90	3.23 ± 0.90
HDL cholesterol (mmol/L)		
Men	1.11 ± 0.29	1.12 ± 0.30
Women	1.46 ± 0.40	1.48 ± 0.41
Ratio of LDL to HDL cholesterol		
Men	3.14 ± 1.09	3.12 ± 1.07
Women	2.37 ± 0.95	2.40 ± 1.07
Apolipoprotein A-I (g/L)		
Men	1.41 ± 0.27	1.43 ± 0.22
Women	1.62 ± 0.26	1.64 ± 0.26
Apolipoprotein B (g/L)		
Men	1.17 ± 0.66	1.16 ± 0.25
Women	1.09 ± 0.28	1.09 ± 0.27
Triacylglycerol (mmol/L)		
Men	1.78 ± 1.39	1.76 ± 1.24
Women	1.48 ± 0.85	1.49 ± 1.14
Saturated fat intake (% of energy)		
Men	10.7 ± 2.8	11.1 ± 3.2
Women	10.3 ± 2.8	10.7 ± 3.1
Monounsaturated fat intake (% of energy)		
Men	11.6 ± 2.5	11.8 ± 2.7
Women	10.9 ± 2.7	11.2 ± 2.6
Polyunsaturated fat intake (% of energy)		
Men	5.7 ± 1.7	5.8 ± 2.0
Women	5.9 ± 1.7	6.1 ± 1.7

[1] $\bar{x}$ ± SD. There were no significant differences between *APOA1* genotypes. Sample sizes were as follows: 530 men (70.2%) and 581 women (70.7%) had the *G/G* genotype and 225 men (29.8%) and 241 women (29.3%) had the *G/A* or *A/A* genotypes.

HDL-cholesterol concentrations in women (B = 0.007 mmol/L for the carriers of the *A* allele, in comparison with the *G/G* homozygotes; NS). In model 2, we added total fat intake (expressed as a percentage of energy in continuous form) and its interaction term with the *APOA1* genotype to model 1. This interaction term was not significant. For models 3 and 4, we started with model 1 and added SFA and MUFA intakes, respectively, along with the corresponding interaction terms with the *APOA1* genotype. The *P* values for these interaction terms remained nonsignificant.

Finally, for model 5, we added PUFA intake to model 1, along with its interaction term with *APOA1* genotype, which was significantly associated with HDL-cholesterol concentration (*P* = 0.005). According to this model, female carriers of the *A* allele had lower HDL-cholesterol concentrations than did *G/G* homozygotes (*P* = 0.008); mean values were lower by an average of 0.274 ± 0.104 mmol/L. However, this unadjusted genetic effect was modulated significantly by PUFA intake. The regres-

sion equation suggests that for every 1% increase in PUFA intake, the HDL-cholesterol concentration increases 0.046 mmol/L (95% CI: 0.014, 0.079) in female carriers of the *A* allele as compared with *G/G* homozygotes (*P* = 0.005). To show this effect in a cumulative form, the subjects were divided into 2 groups with higher and lower PUFA intakes (ie, above and below the mean for the subject population). When the continuous PUFA-intake variable in model 5 was replaced with the dichotomous form (PUFA intake ≤6% and >6% of energy), its interaction term with the *APOA1* genotype remained statistically significant (*P* = 0.049). In women who consumed <6% of energy from PUFA, mean HDL-cholesterol concentrations for *G/G* homozygotes and carriers of the *A* allele were 1.49 ± 0.40 and 1.44 ± 0.40 mmol/L, respectively. In women who consumed >6% of energy from PUFA, these values were 1.44 ± 0.39 and 1.49 ± 0.39 mmol/L, respectively.

For men, the results of the same type of linear regression analysis are shown in **Table 4**. The magnitude of the regression coefficient for the *APOA1* genotype was greater when dietary fat was added to the model, but none of the interactions between fat intake and the *APOA1* genotype in men were statistically significant. When the continuous PUFA-intake variable was made into a dichotomous variable, the interaction term between this variable and the *APOA1* genotype was also nonsignificant in men. Furthermore, no significant differences in HDL-cholesterol concentrations were found between the 2 groups with higher and lower PUFA intakes.

Finally, we tested the heterogeneity of the modulation by PUFA intake of the effects of the *APOA1* G-A polymorphism on HDL-cholesterol concentrations in men and women. To do this, we fitted a hierarchical multivariate regression model including the interaction term between sex, *APOA1* genotype, and PUFA intake. The significance of this sex × gene × diet interaction term (*P* = 0.044) supported the heterogeneity of the PUFA-intake effect.

The correlation coefficients between PUFA intake and MUFA intake were *r* = 0.54 and *r* = 0.51 in women and men, respectively. Between PUFA and SFA intakes, the correlations were *r* = 0.22 in women and *r* = 0.14 in men. Because these correlations were not excessively high, we were able to retain these variables in the multivariate regression models without problems of colinearity and we also could obtain a more independent estimate of the regression coefficient for PUFA intake. Thus, when model 5 in Tables 3 and 4 was additionally adjusted for SFA intake and MUFA intake (model 6, not shown), the interaction term between *APOA1* genotype and PUFA intake remained significant in women (B = 0.045; *P* = 0.007) but was not significant in men (B = 0.002; *P* = 0.86). In men, we observed a significant 4-way interaction (*P* = 0.023) between *APOA1* genotype, PUFA intake, tobacco smoking, and alcohol consumption. However, this study did not have enough statistical power to analyze this effect within each of the 2 categories for PUFA intakes (ie, high and low), tobacco smoking (smokers and nonsmokers), or alcohol consumption (drinkers and nondrinkers).

Dietary fat intakes, *APOA1* alleles, and apo A-I concentrations

Next we examined apo A-I concentrations as the dependent variable. Multivariate regression models 1–6 were fitted as previously described, for men and women separately (results not shown). In women, the *P* values for the interaction terms with *APOA1* alleles were all not significant. Although the interaction term between the *APOA1* genotype and PUFA intake was not

TABLE 3
Interaction of fat consumption with the effects of *APOA1* alleles on HDL–cholesterol concentrations (in mmol/L), with control for age, BMI, alcohol consumption, and tobacco smoking: multiple linear regression analysis in women[1]

	Model 1: core model		Model 2: model 1 + total fat intake		Model 3: model 1 + SFA intake		Model 4: model 1 + MUFA intake		Model 5: model 1 + PUFA intake	
	B	P	B	P	B	P	B	P	B	P
APOA1 genotype (*G/A + A/A* or *G/G*)[2]	0.007 (0.028)	0.797	−0.194 (0.132)	0.146	−0.041 (0.104)	0.691	−0.179 (0.124)	0.148	−0.274 (0.104)	0.008
Alcohol (nonconsumption or consumption)	−0.119 (0.028)	<0.001	−0.124 (0.028)	<0.001	−0.127 (0.028)	<0.001	−0.123 (0.029)	<0.001	−0.113 (0.028)	<0.001
Tobacco (smoking or nonsmoking)	−0.122 (0.032)	<0.001	−0.140 (0.033)	<0.001	−0.142 (0.034)	<0.001	−0.134 (0.034)	<0.001	−0.123 (0.034)	<0.001
BMI (kg/m²)	−0.028 (0.002)	<0.001	−0.029 (0.002)	<0.001	−0.029 (0.002)	<0.001	−0.029 (0.002)	<0.001	−0.028 (0.002)	<0.001
Total fat intake (% of energy)			0.004 (0.007)	0.099						
Interaction (total fat × *APOA1*)[3]			0.007 (0.004)	0.130						
SFA intake (% of energy)					0.012 (0.006)	0.028				
Interaction (SFA × *APOA1*)					0.004 (0.010)	0.653				
MUFA intake (% of energy)							0.007 (0.006)	0.220		
Interaction (MUFA × *APOA1*)							0.017 (0.011)	0.128		
PUFA intake (% of energy)									−0.005 (0.009)	0.605
Interaction (PUFA × *APOA1*)									0.046 (0.013)	0.005
R² of the model[4]	0.17	<0.001	0.19	<0.001	0.18	<0.001	0.18	<0.001	0.19	<0.001

[1] B, regression coefficient; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
[2] SE in parentheses.
[3] *APOA1* refers to the genotype (*G/G* compared with *G/A + A/A* combined).
[4] Additionally adjusted for age and energy intake.

TABLE 4
Interaction of fat consumption with the effects of *APOA1* alleles on HDL–cholesterol concentrations (in mmol/L), with control for age, BMI, alcohol consumption, and tobacco smoking: multiple linear regression analysis in men[1]

	Model 1: core model		Model 2: model 1 + total fat intake		Model 3: model 1 + SFA intake		Model 4: model 1 + MUFA intake		Model 5: model 1 + PUFA intake	
	B	P	B	P	B	P	B	P	B	P
APOA1 genotype (*G/A + A/A* or *G/G*)[2]	0.017 (0.022)	0.452	0.077 (0.102)	0.454	0.072 (0.082)	0.382	0.088 (0.097)	0.362	−0.001 (0.072)	0.994
Alcohol (nonconsumption or consumption)	−0.160 (0.025)	<0.001	−0.171 (0.026)	<0.001	−0.173 (0.026)	<0.001	−0.171 (0.026)	<0.001	−0.161 (0.025)	<0.001
Tobacco (smoking or nonsmoking)	−0.028 (0.026)	0.275	−0.032 (0.026)	0.210	−0.039 (0.026)	0.140	−0.032 (0.026)	0.212	−0.029 (0.026)	0.254
BMI (kg/m²)	−0.016 (0.002)	<0.001	−0.016 (0.002)	<0.001	−0.016 (0.002)	<0.001	−0.016 (0.002)	<0.001	−0.016 (0.002)	<0.001
Total fat intake (% of energy)			0.004 (0.002)	0.063						
Interaction (total fat × *APOA1*)[3]			−0.002 (0.003)	0.533						
SFA intake (% of energy)					0.011 (0.004)	0.013				
Interaction (SFA × *APOA1*)					−0.005 (0.007)	0.456				
MUFA intake (% of energy)							0.008 (0.005)	0.100		
Interaction (MUFA × *APOA1*)							−0.013 (0.008)	0.439		
PUFA intake (% of energy)									−0.004 (0.007)	0.410
Interaction (PUFA × *APOA1*)									0.003 (0.012)	0.799
R² of the model[4]	0.10	<0.001	0.11	<0.001	0.11	<0.001	0.10	<0.001	0.10	<0.001

[1] B, regression coefficient; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
[2] SE in parentheses.
[3] *APOA1* refers to the genotype (*G/G* compared with *G/A + A/A* combined).
[4] Additionally adjusted for age and energy intake.

TABLE 5
Plasma HDL-cholesterol and apolipoprotein A-I concentrations by *APOA* genotype, polyunsaturated fatty acid (PUFA) intake, and sex

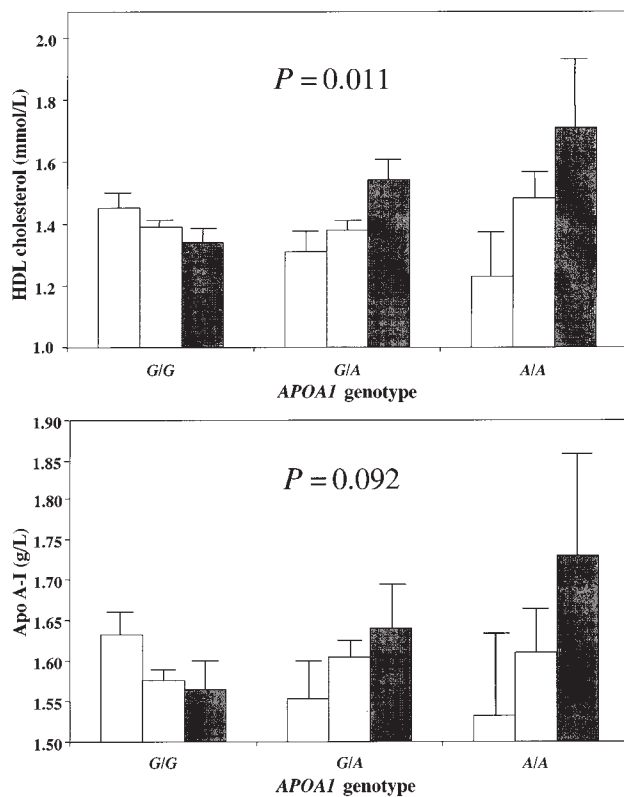| | PUFA intake as % of energy intake | | | | |
	≤4% (n = 107 F, 118 M)	4–8% (n = 624 F, 585 M)	>8% (n = 91 F, 52 M)	*P* for trend[1]	*P* for interaction[2]
Women					
HDL cholesterol (mmol/L)					0.012
G/G	1.51 ± 0.43[3]	1.46 ± 0.40	1.40 ± 0.36	0.085	
G/A + A/A	1.32 ± 0.35	1.48 ± 0.40	1.58 ± 0.48	0.015	
Unadjusted *P*[4]	0.027	0.713	0.048		
Adjusted *P*[5]	0.041	0.795	0.049		
Apolipoprotein A-I (mmol/L)					0.079
G/G	1.68 ± 0.27	1.62 ± 0.25	1.60 ± 0.25	0.086	
G/A + A/A	1.56 ± 0.24	1.65 ± 0.25	1.65 ± 0.33	0.220	
Unadjusted *P*[4]	0.038	0.157	0.499		
Adjusted *P*[5]	0.153	0.177	0.512		
Men					
HDL cholesterol (mmol/L)					0.653
G/G	1.14 ± 0.32	1.11 ± 0.28	1.11 ± 0.25	0.604	
G/A + A/A	1.18 ± 0.39	1.11 ± 0.28	1.22 ± 0.33	0.699	
Apolipoprotein A-I (mmol/L)					0.408
G/G	1.45 ± 0.22	1.41 ± 0.21	1.40 ± 0.19	0.236	
G/A + A/A	1.44 ± 0.28	1.42 ± 0.20	1.54 ± 0.30	0.129	

[1] ANOVA for the global comparison of the 3 PUFA-intake categories within genotypes.

[2] Interaction of *APOA1* genotype × PUFA intake (3 categories) in the linear regression model adjusted for age, BMI, alcohol intake, tobacco smoking, and intakes of polyunsaturated, saturated, and monounsaturated fatty acids.

[3] $\bar{x}$ ± SD.

[4] Unadjusted comparison between *G/G* subjects and *G/A + A/A* subjects combined.

[5] Comparison between *G/G* subjects and *G/A + A/A* subjects combined, adjusted for age, BMI, tobacco smoking, alcohol consumption, and intakes of saturated, monounsaturated, and polyunsaturated fatty acids.

significant for apo A-I concentrations in women, the effects were in the same direction as those observed for HDL-cholesterol concentrations. No significant interactions with smoking or alcohol consumption were observed in women. In men, the *P* values for the *APOA1* × fat intake interactions were not significant for total fat, MUFA, SFA, and PUFA intakes. When alcohol and smoking interactions were also considered in model 6, a significant 3-way interaction (*P* = 0.035) between PUFA intake, alcohol consumption, and *APOA1* genotype was found. The effect of this interaction was as follows: carriers of the *A* allele had lower concentrations of apo A-I than did *G/G* homozygotes, but apo A-I concentrations were higher with higher PUFA intakes in a linear pattern that differed between drinkers and nondrinkers. The slope of the line was higher in nondrinkers than in drinkers.

We also divided both male and female subjects into 3 categories of PUFA intake (<4% of energy, 4%–8% of energy, and >8% of energy) to obtain mean values that would allow direct comparison with other studies. Mean HDL-cholesterol and apo A-I concentrations by *APOA1* genotype and sex across the 3 categories of PUFA intake are shown in **Table 5**. *P* values are shown for the unadjusted comparisons and for comparisons adjusted for age, BMI, alcohol consumption, tobacco smoking, and fat intake. In women, *APOA1* genotype clearly had a different effect across the categories of PUFA intake. When PUFA intake was low, *G/G* homozygotes had ≈14% higher HDL-cholesterol concentrations than did carriers of the *A* allele (*P* < 0.05). When PUFA intake was high, HDL-cholesterol concentrations in carriers of the *A* allele were 13% higher on average than were

concentrations in the *G/G* group. For apo A-I concentrations, the trend was not different from that observed for HDL cholesterol, but it was not significant. In men, an additional stratification by alcohol consumption or tobacco smoking was necessary to obtain significant differences between *G/G* homozygotes and A carriers (data not shown).

Finally, to test a gene-dosage effect of the *APOA1* genotype × PUFA intake interaction in women, the group of carriers of the *A* allele was divided into the *G/A* and *A/A* genotypes. In **Figure 1**, the estimated mean HDL-cholesterol and apo A-I concentrations in *G/G*, *G/A*, and *A/A* women across the 3 categories of PUFA intake are shown. These means were adjusted for age, BMI, tobacco smoking, alcohol consumption, SFA intake, and MUFA intake. The *P* values shown are for the interaction between the *APOA1* genotype and the 3 categories of PUFA intake. For HDL cholesterol, this interaction was significant and a clear gene-dosage effect was observed: subjects with the *A/A* genotype had the highest HDL-cholesterol concentrations in the category of PUFA intake >8% of energy and the lowest HDL-cholesterol concentrations in the category of PUFA intake <4% of energy. Moreover, when PUFA intake was analyzed as a continuous variable, the regression coefficient for *A/A* subjects adjusted for all covariates was 2.5 times higher than that of *G/A* heterozygotes (*P* < 0.05). In the regression model for apo A-I concentrations, we observed a gene-dosage effect similar to that for HDL cholesterol, but the effect was not significant. In men, the gene-dosage effect was not tested because of the higher-order interactions and limited sample size.

FIGURE 1. Mean (±SE) HDL-cholesterol and apolipoprotein (apo) A-I concentrations by *APOA1* genotype and polyunsaturated fatty acid (PUFA) intake categories (□, <4%; ▨, 4%–8%; ■, >8% of energy) in women. Means were adjusted for age, body mass index, alcohol consumption, tobacco smoking, and intakes of energy, saturated fatty acids, monounsaturated fatty acids, and PUFAs. The *P* values shown were obtained for the interaction between *APOA1* genotype and PUFA intake in the multivariate linear regression models.

DISCUSSION

The association of the G-to-A substitution at position −75 of the *APOA1* gene promoter with plasma HDL-cholesterol concentrations has been examined extensively since the early study in the United Kingdom (8) showing that men with the *A* allele had HDL and apo A-I concentrations that were significantly higher than those of *G/G* homozygotes. During the same year, Pagani et al (9) reported that in Italian women, the frequency of the *A* allele increased from the lowest to the highest decile of HDL-cholesterol concentrations, but no such trend was detected in men. These promising observations raised considerable interest and the *APOA1* G-A polymorphism was subsequently studied by several other groups. Associations between the *A* allele and elevated HDL-cholesterol or apo A-I concentrations were reported in Italian boys, but not girls (12); in healthy, physically active boys and young males from Belgium (11); in Finnish men (10); and in French-Canadian women (16). In contrast, no associations of the *A* allele with HDL-cholesterol or apo A-I concentrations were reported by Civeira et al (18), Lopez-Miranda et al (26), Mata et al (27), Carmena-Ramon et al (28), Akita et al (20), or Barre et al (19). Moreover, Matsunaga et al (24) observed that control subjects with the *G/A* genotype had significantly lower plasma concentrations of apo A-I. The inconsistency of these findings suggests that some of the observed associations could be the result of chance, especially considering the small sample sizes of several of the studies. Alternatively, the effects of the −75 bp G-A polymorphic site could be dependent on environmental factors that differ between study populations. This hypothesis gained support when Sigurdsson et al (15) reported that the *A* allele was only associated with increased HDL-cholesterol or apo A-I concentrations in nonsmoking men. This gene-environment interaction was subsequently confirmed by a similar finding in Chinese subjects residing in Singapore (13). These findings are also consistent with the failure to detect significant associations in those studies with a high prevalence of smokers (18). Although the evidence suggests that smoking status may interact with the effect of genotype, many of the inconsistencies remain unexplained.

Some investigators reported that the G-A polymorphism is associated with variability in LDL-cholesterol or apo B concentrations rather than HDL-related variables (12, 27, 28). These findings provide some support for the alternative hypothesis that this mutation is not causative, but is in linkage disequilibrium with another functional mutation in one of the neighboring genes (16). Moreover, previous studies showed significant interactions between the G-A polymorphism and LDL-cholesterol response to different types of dietary intervention (26, 27), although other studies found no significant interactions (10, 28). Note that the gene-diet interactions for LDL-cholesterol response observed in the short-term dietary intervention studies (26, 27) were not significant when tested in the present population-based study examining long-term dietary habits. However, the trends in the present study (data not shown) were in the same direction as those previously reported (26, 27).

To address our primary goal, we examined whether fat intake is one of the factors that modify the association between the G-A polymorphism and HDL-cholesterol or apo A-I concentrations. Our study showed a significant interaction between dietary PUFA intake and the G-A polymorphism on plasma HDL-cholesterol and apo A-I concentrations. In general, our model showed that the *A* allele was associated with lower concentrations of HDL cholesterol and apo A-I. However, in subjects carrying the *A* allele, a higher PUFA intake may reverse the genetic effect. In women, this interaction was highly significant for HDL-cholesterol concentrations and approached significance for apo A-I concentrations. The complexity of this gene-diet interaction differed between men and women. In men, the PUFA-intake effect was only significant when interactions with alcohol consumption and tobacco smoking were also considered in the regression models (data not shown).

We propose that, in addition to the previously reported genotype-smoking interaction, the gene-diet interaction studied here could help explain some of the contradictory findings reported previously. According to our statistical model, a moderate consumption of PUFAs (4%–8% of total energy), common in most populations, would be consistent with the absence of significant genotype-phenotype associations as reported by several studies. An association between the *G/G* genotype and higher HDL-cholesterol concentrations would be expected in subjects consuming <4% of energy from PUFAs. However, such low PUFA intakes are uncommon in most populations, and only one study observed higher apo A-I concentrations in *G/G* homozygotes than in carriers of the *A* allele (24). Conversely, it would be expected that the *A* allele would be associated with significantly higher HDL-cholesterol concentrations in sub-

jects consuming >8% of their daily energy intake as PUFAs than in subjects consuming lower amounts of PUFAs. Distinguishing the associations of various types of dietary fat with blood lipid concentrations in an observational study is difficult because of the multicolinearity of fat intakes. In the present study, separate multivariate regression models were computed for the different types of fat. Only PUFA intake showed significant results. When SFA and MUFA intakes were included in the PUFA regression model, the regression coefficient for PUFA intake remained significant.

The role of dietary fatty acids in regulating plasma lipoprotein concentrations is well documented (44). Compared with SFA intake, PUFA intake was shown to lower LDL- and HDL-cholesterol concentrations (45). However, these studies did not address genetic variability in response. When we considered the G-A polymorphism in our study, the lowering effect of PUFA intake on HDL-cholesterol concentrations was seen only in *G/G* subjects; the opposite effect was seen in carriers of the *A* allele. This observation, which requires further investigation in intervention studies, may be of particular relevance for dietary counseling designed to improve the lipid profile and thereby reduce the risk of atherosclerosis. However, we should keep in mind that other factors, such as BMI, alcohol intake, and physical activity, are also major modulators of HDL-cholesterol concentrations, response to diet, and genotype or phenotype associations (27, 46–49).

Dietary PUFAs can induce gene expression, as was shown in animal models and cultured cells (29). This polymorphic site, variably denoted as −75 bp, −76 bp, or −78 bp in different reports, is located in the 5′ flanking region of the transcriptional start site of the apo A-I gene. Studies that used the more common *G* allele have not identified proteins binding at this specific site (33). However, a drug-responsive element was localized in the immediate neighborhood (50). In addition, the presence of the A substitution creates a 6-bp repeat that has homology to nuclear binding sites. Moreover, this repeat may allow the formation of a DNA secondary structure, which could interfere with protein interaction in the transcriptional apparatus. This agrees with results from some of the in vitro studies, which showed that the *A* allele was associated with lower in vitro transcriptional efficiency (30–32). Further evidence in support of this mutation and *APOA1* gene transcription was provided by in vivo metabolic studies showing that subjects with the *A* allele had lower apo A-I production rates (31). These data are also consistent with our model, suggesting that under basal conditions the *A* allele is associated with lower HDL-cholesterol and apo A-I concentrations. Thus, PUFAs could interact differently with the transcriptional apparatus of the *APOA1* gene, increasing transcription only in the presence of the *A* allele.

Another important finding of this research relates to the sex differences observed for genotype-PUFA interactions, which suggests a hormonal effect. Thyroid hormones, glucocorticoids, and estradiol enhance activity of the *APOA1* gene, whereas retinoic acid and androgens decrease it (51, 52). Specifically, regulation of the *APOA1* gene by estrogen may vary in terms of direction and magnitude depending not only on the presence of estrogen receptor-α and 17β-estradiol, but also on the intracellular balance of estrogen receptor-α and coactivators used by estrogen receptor-α and the *APOA1* enhancer (53). We hypothesized that the G-A polymorphism may be implicated in this mechanism. Alternatively, this mutation could be in linkage disequilibrium with a functional mutation in the promoter or enhancer regions of the *APOA1* gene.

Although we cannot rule out the possibility of confounding in our PUFA hypothesis, it is unlikely that confounding would fully explain the strong interaction observed. The lowering effect of the *A* allele on HDL-cholesterol concentrations in women also showed a clear gene-dosage relation when the interaction with PUFAs was considered. *A/A* subjects had lower HDL-cholesterol concentrations than did heterozygous *G/A* individuals. Moreover, the elevating effect of PUFA intake on HDL-cholesterol concentrations was greater in *A/A* subjects than in *G/A* subjects.

In summary, PUFA intake modulates the effect of the G-A polymorphism in the *APOA1* gene promoter, resulting in higher concentrations of HDL cholesterol in individuals carrying the *A* allele. According to these results, the known HDL-cholesterol-lowering effect attributed to the consumption of PUFAs does not apply to women carrying the *A* allele. Furthermore, this specific population group might benefit from a high-PUFA diet, which should increase HDL-cholesterol concentrations and thereby reduce CHD risk. Our results illustrate the complexity of polymorphism-phenotype associations and underscore the importance of accounting for interactions between genes and environmental factors in population genetic studies.

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