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Dietary Intake of n-6 Fatty Acids Modulates Effect of Apolipoprotein A5 Gene on Plasma Fasting Triglycerides, Remnant Lipoprotein Concentrations, and Lipoprotein Particle Size

The Framingham Heart Study

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Background—Apolipoprotein A5 gene (*APOA5*) variation is associated with plasma triglycerides (TGs). However, little is known about whether dietary fat modulates this association.

Methods and Results—We investigated the interaction between *APOA5* gene variation and dietary fat in determining plasma fasting TGs, remnant-like particle (RLP) concentrations, and lipoprotein particle size in 1001 men and 1147 women who were Framingham Heart Study participants. Polymorphisms $-1131T>C$ and $56C>G$, representing 2 independent haplotypes, were analyzed. Significant gene–diet interactions between the $-1131T>C$ polymorphism and polyunsaturated fatty acid (PUFA) intake were found ($P<0.001$) in determining fasting TGs, RLP concentrations, and particle size, but these interactions were not found for the $56C>G$ polymorphism. The $-1131C$ allele was associated with higher fasting TGs and RLP concentrations ($P<0.01$) in only the subjects consuming a high-PUFA diet ($>6\%$ of total energy). No heterogeneity by sex was found. These interactions showed a dose-response effect when PUFA intake was considered as a continuous variable ($P<0.01$). Similar interactions were found for the sizes of VLDL and LDL particles. Only in carriers of the $-1131C$ allele did the size of these particles increase (VLDL) or decrease (LDL) as PUFA intake increased ($P<0.01$). We further analyzed the effects of n-6 and n-3 fatty acids and found that the PUFA–*APOA5* interactions were specific for dietary n-6 fatty acids.

Conclusions—Higher n-6 (but not n-3) PUFA intake increased fasting TGs, RLP concentrations, and VLDL size and decreased LDL size in *APOA5* $-1131C$ carriers, suggesting that n-6 PUFA-rich diets are related to a more atherogenic lipid profile in these subjects. (*Circulation*. 2006;113:2062-2070.)

Key Words: diet ■ fatty acids ■ genetics ■ lipids ■ lipoproteins

The impact of hypertriglyceridemia on coronary heart disease risk has long been a matter of debate; however, evidence supporting the prognostic value of this lipid fraction has been gained during the past decade.^{1,2} Triglyceride (TG)-rich lipoproteins comprise a great variety of nascent and metabolically modified lipoprotein particles, and they are especially relevant during the postprandial state.³ Remnant lipoproteins have also been shown to promote atherogenesis,^{4–6} and plasma remnant-like particles (RLPs) can be measured by an antibody-based assay.⁷ In the Framingham Offspring Study,⁸ RLPs were significantly increased in women with diabetes and in men compared with control subjects without diabetes.

Clinical Perspective p 2070

Apolipoprotein A5 (*APOA5*)^{9,10} is found preferentially on HDL but is thought to transfer to VLDL during the postprandial state.¹¹ Despite its very low levels in plasma, *APOA5* is an important regulator of TG-rich lipoprotein (TRL) metabolism.¹² Two mechanisms have been proposed. The first suggests a role of *APOA5* in VLDL assembly^{13,14}; the second supports the possibility that *APOA5* may be an activator of intravascular TG hydrolysis by lipoprotein lipase (LPL).^{15–17} However, both proposals are not mutually exclusive, and these mechanisms may be

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working simultaneously or alternatively, depending on specific metabolic situations.

Several common *APOA5* single-nucleotide polymorphisms (SNPs) have been associated with increased plasma total TG, RLP, and VLDL concentrations.^{18–22} Moreover, current knowledge suggests an association between *APOA5* and postprandial lipemia that could be modulated by the type of dietary fat.^{23,24} In this regard, polyunsaturated fatty acids (PUFAs) are known modulators of gene expression.²⁵ Along these lines, we have reported that dietary PUFAs interact with the leucine to valine (L162V) substitution at the peroxisome proliferator-activated receptor- α (PPAR- α) gene in determining plasma TG and apolipoprotein C3 concentrations in the Framingham Study participants.²⁶ Considering that PPAR- α is a nuclear transcription factor regulating multiple genes involved in lipid metabolism (including *APOA5*²⁷), our previous results prompted us to examine the hypothesis that PUFA intake may modulate the effect of *APOA5* variants on lipid metabolism. Moreover, given the different cardiovascular protection attributed to n-6 and n-3 PUFAs,²⁸ we examined their potential interactions with *APOA5* variants in a large population-based study, the Framingham Heart Study.

Methods

Study Design and Subjects

The study sample consisted of 2148 subjects who participated in the Framingham Offspring Study.²⁹ Lipids, other cardiovascular disease (CVD) risk factors, and dietary intake were recorded for subjects who participated in the fifth examination, conducted between 1992 and 1995 (n=3515). DNA was obtained from 1987 to 1991. The Institutional Review Board for Human Research at Boston University and Tufts University/New England Medical Center approved the protocol of the present study. All participants provided written informed consent.

The present study included only subjects with phenotypic data and complete dietary information for whom *APOA5* gene variants were examined. Subjects taking lipid-lowering medications as well as subjects with any missing data on control variables (age, body mass index [BMI], smoking, alcohol consumption, diabetes status, β -blocker use, and estrogen use in women) were excluded from our analyses. Thus, data for 1001 men and 1147 women who met the above criteria were analyzed. Because nearly all subjects were white, no control for ethnicity was needed. Although in the Framingham Study recruitment of families was planned,²⁹ in this specific cycle most participants were unrelated, and the number of individuals within each family included in the present study was very low. Thus, the 1147 women considered in the present study were distributed in 893 pedigrees, and 752 (65%) were singletons. In the nonsingletons, 113 women were unrelated, and the others were mostly siblings and cousins. The 1001 men in the present study were distributed in 814 pedigrees, and 697 of the 1001 (\approx 70%) were singletons. In the families, 53 men were unrelated, and the others were mostly siblings and cousins.

Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes by standard methods. *APOA5* SNPs -1131T>C(rs662799), -3A>G(rs651821), IVS3+476G>A(rs2072560), and 1259T>C(rs2266788) and 56C>G(rs3135506) were genotyped as previously described^{21,22} by use of the ABI Prism SNaPshot multiplex system (Applied Biosystems, Foster City, Calif).

Measurement of Plasma Lipid, Lipoprotein, and Apolipoprotein

The standardized procedures for sample collection and biochemical analysis for TGs, total cholesterol, LDL cholesterol, and HDL

cholesterol have been described previously.³⁰ VLDL and LDL subclass distributions were determined by proton nuclear magnetic resonance spectroscopy.³¹ Measurements of RLP-TG and RLP cholesterol (RLP-C) concentrations have previously been described.⁷

Dietary Assessment

Dietary intake was estimated with the semiquantitative Willett food-frequency questionnaire (Rimm et al³²), which specified serving sizes. This questionnaire has been validated for total PUFA intake as well as n-3 and n-6 fatty acids (FAs).^{32,33} Intakes of total fat, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), total PUFAs, and n-3 and n-6 PUFAs were expressed as percentages of total energy and were included in analyses as both continuous and categorical variables. To construct categorical variables, intakes were classified into 2 groups according to the mean value of the population (ie, groups had intakes below and above the mean). In addition, we considered together as n-3 PUFA the dietary intake of α -linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid. Linoleic acid and arachidonic acid were considered together as n-6 PUFA. Tertiles of PUFA intakes (n-6 and n-3) were also considered.

Statistical Analyses

We examined all continuous variables for normality of distribution. TG, RLP-TG, and RLP total cholesterol (RLP-C) concentrations were log-transformed. Results were presented as the antilog of the estimated log values. The relationship between *APOA5* genotypes, dietary PUFAs, and plasma lipid-related measures was evaluated by analysis of covariance techniques. Because the present study involved some correlated data that were due to familial relationships (siblings and cousins), we controlled for familial relationships. We used 2 approaches to accomplish these analyses. First, a generalized linear mixed-model approach, which assumed an exchangeable correlation structure among all members of a family (PROC MIXED in SAS, Cary, NC), was used. Second, because this approach could not accurately represent the true correlation structure within these pedigrees, we used a measured-genotype approach as implemented in SOLAR, a variance component-analysis computer package for quantitative traits measured in pedigrees of arbitrary size.³⁴ In these analyses, we used several different models to adjust for potential confounders. After having checked that the results obtained using the generalized mixed model were similar to those of the SOLAR approach because of the large number of unrelated subjects in this sample, we decided to use the generalized mixed model for the adjustment of familial relationships. The interactions between dietary PUFAs (as a continuous or as a categorical variable) and the *APOA5* polymorphisms were tested in a hierarchical multivariate-interaction model after controlling for potential confounders, including sex, age, BMI, smoking, alcohol consumption, total energy, fat and carbohydrate intakes, diabetes status, β -blocker use, menopausal status, and estrogen use (in women). These analyses were performed for the whole sample and for men and women separately in order to verify the homogeneity and the magnitude of the effect. Standard regression diagnostic procedures, including multicollinearity tests, homogeneity of variance tests, normal plots of the residuals, and residuals by Student *t* test, were used to ensure the appropriateness of these models. The population means for PUFA intake and for other types of fat were considered the cutoff points to dichotomize these variables. When PUFA intake was considered as a continuous variable, its interaction with the *APOA5* polymorphism was depicted by computing the predicted values for each individual from the adjusted regression model and plotting these values against PUFA intake depending on the *APOA5* genotype. The haplotype analysis was carried out as previously described.²² The pairwise linkage disequilibria (LDs) between SNPs at the *APOA5* locus were estimated as correlation coefficient *R*, with the HelixTree program using unrelated subjects. Inference and possible haplotypes were computed via an expectation-maximization algorithm.²² All reported statistical tests were 2 sided. To minimize type I errors, which are increased because of the high number of comparisons, only values of $P \leq 0.01$ were considered statistically significant.

TABLE 1. Demographic, Biochemical, Dietary, and Genotypic Data

Characteristics of Participants According to Sex	Men (n=1001)	Women (n=1147)
Age, y	50.8±9.9	50.1±9.6
BMI, kg/m ²	27.6±3.8	25.9±5.5
LDL-C, mg/dL	135±33	127±35
HDL-C, mg/dL	44±11	56±15*
TG, mg/dL	135±97	105±101*
RLP-TG,† mg/dL	30.7±45.0	22.2±70.2*
RLP-C,† mg/dL	8.54±6.79	7.13±6.31*
LDL diameter, nm	20.69±0.59	21.08±0.46
VLDL diameter, nm	48.28±9.24	44.20±8.58*
Glucose, mg/dL	97.0±23.0	91.1±19.3*
Energy intake, kcal/d	2004±643	1745±576*
Total fat, g/d	67.1±27.6	56.9±23.8*
SFA, % of energy	10.6±2.9	10.3±2.9*
MUFA, % of energy	11.5±2.6	11.0±2.6*
n-6 PUFA, % of energy	5.1±1.5	5.2±1.6
n-3 PUFA, % of energy	0.65±0.2	0.71±0.2*
PUFA, % of energy	5.8±1.6	6.0±1.7
Animal fat, % of energy	15.9±5.5	15.1±5.1*
Vegetable fat, % of energy	14.0±4.4	14.1±4.5
Carbohydrate, % of energy	50.2±8.5	51.9±8.4
Fiber, g/d	19.1±8.2	19.2±8.4
Alcohol, g/d	14.7±19.4	7.2±11.8*
Drinkers, n (%)	751 (75)	768 (67)*
Smokers, n (%)	233 (23.3)	247 (21.6)
β-Blocker treatment, n (%)	111 (11.1)	73 (6.3)*
Diabetes status, n (%)	74 (7.4)	44 (3.8)
On estrogen treatment, n (%)	0 (0)	90 (7.9)
<i>APOA5</i> -1131T>C,‡ n (%)		
TT	816 (86.2)	936 (87.0)
C carriers	131 (13.8)	140 (13.0)
<i>APOA5</i> 56C>G,‡ n (%)		
CC	860 (87.5)	1002 (89.1)
G carriers	123 (12.5)	123 (11.0)

Values are mean±SD or n (%).

*Significantly different from men ($P<0.01$).

†Data for these variables were available for only a random sample of these participants (n=1600).

‡*APOA5* genetic data was successfully obtained in 947 men and 1076 women for the -1131T>C polymorphism and for 983 men and 1125 women for the 56C>G polymorphism.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Information about demographic, biochemical, dietary intake, and genotypic data are provided in Table 1. Five *APOA5* SNPs (-1131T>C, -3A>G, 56C>G, IVS3+476G>A, and 1259T>C) were analyzed. Genotype

frequencies did not deviate from Hardy-Weinberg equilibrium expectations. Allele frequencies for the rare alleles listed above were 0.069, 0.071, 0.059, 0.064, and 0.072, respectively. As previously shown,²² 4 of the *APOA5* SNPs (-1131T>C, -3A>G, IVS3+476G>A, and 1259T>C) were in strong LD with each other and shared the same haplotype. Pairwise LD coefficient R values between the -1131T>C SNP and SNPs -3A>G, IVS3+476G>A, and 1259T>C in unrelated subjects were 1, 1, and 1, respectively ($P<0.001$). The haplotype defined by these SNPs was named haplotype *APOA5**2.¹⁸ In contrast, SNP 56C>G was independent of the aforementioned SNPs, representing a different haplotype (ie, *APOA5**3).¹⁸ Pairwise LD coefficient R between the -1131T>C SNP and 56C>G was 0.063. Furthermore, we investigated gene-diet interactions determining plasma TGs, TRL concentrations, and lipoprotein particle sizes for each of the 5 SNPs as well as for the most prevalent haplotypes (11111 [representing 86.3% of all haplotypes] and 22122 and 11211 [accounting for 6.2% and 5.7%, respectively]). As expected, the analyses of each of the 4 SNPs in strong LD revealed the same interactions with the dietary PUFA intake on the variables examined, whereas no significant interactions with fat intake were observed for SNP 56C>G (data not shown). Accordingly, in the haplotype association analysis, we obtained statistically significant interactions ($P<0.01$) between PUFA intake and the 22122 haplotype in determining TRL in men and women. No significant interactions were obtained when the 11211 haplotype was analyzed. Because the haplotype analysis did not show better results than did the SNP analysis with the -1131T>C polymorphism, we considered this SNP to be a tag SNP of the *APOA5**2 haplotype.

First, to examine the interaction between -1131T>C SNP and PUFA intake, we dichotomized dietary PUFA according to the population mean ($5.92\pm 1.7\%$, $\approx 6\%$). Homozygotes (CC) and heterozygotes (TC) of the rare allele were combined to increase statistical power. Separate models were fitted for men and women, and although the magnitude of the effect was slightly greater in men, similar directions of effects were observed. Moreover, the homogeneity by sex was also confirmed by examining the probability value of the corresponding interaction term with sex ($P>0.05$ for each parameter examined). Therefore, men and women were analyzed together. After multivariate control for potential confounders, a statistically significant interaction ($P<0.001$) between SNP -1131T>C and PUFA intake ($>6\%$ or $<6\%$ of energy) on TG concentration was found (Figure 1A). In the adjusted model, the -1131C allele was associated with an increase in fasting TG (21%, $P=0.002$) only in subjects consuming $>6\%$ of energy from PUFA. However, mean fasting TG concentrations were not statistically higher in carriers of the -1131C allele compared with the TT homozygotes when the PUFA consumption was low ($P=0.600$). We observed similar and significant interactions between PUFA consumption and SNP -1131T>C on RLP-TG ($P<0.001$) and RLP-C ($P<0.001$). As observed for fasting TGs, concentrations of RLP-TG in

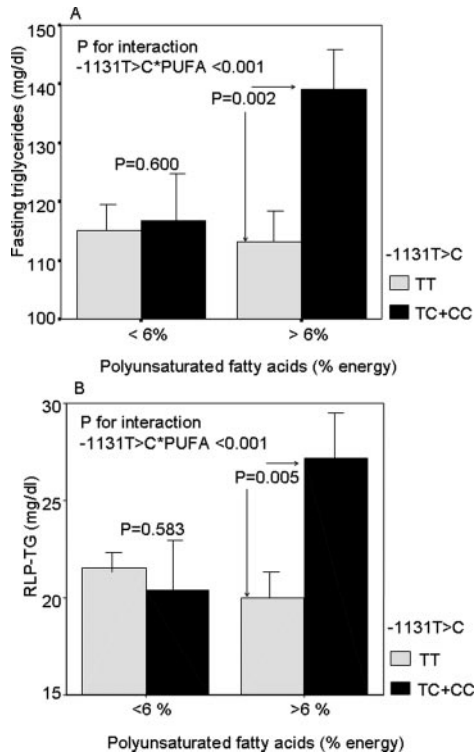


Figure 1. Mean TG (A) and RLP-TG (B) concentrations in men and women combined by *APOA5* -1131T>C genotypes (gray bar indicates TT; black bar, TC+CC) and PUFA intake categories (PUFA <6% and PUFA >6%). Means were adjusted for sex, age, familial relationships, BMI, smoking, alcohol, diabetes status, menopausal status, estrogens, β -blocker use, total fat intake, carbohydrates, and energy. Mean values are antilog of the log value. Bars indicate standard error (SE) of means. SEs were computed to maintain the proportion estimated with the log transformation.

subjects carrying the -1131C allele (Figure 1B) were increased ($\approx 34\%$, $P=0.005$) when they consumed more than 6% of energy from PUFAs. When we analyzed the interaction of SNP -1131T>C and PUFA intake in determining LDL and VLDL sizes, we found statistically significant interactions ($P=0.01$ and $P=0.008$, respectively). These were consistent with a more atherogenic lipid profile in subjects carrying the -1131C allele and consuming more than 6% of energy from PUFAs. Thus, in carriers of the -1131C allele, a high PUFA intake was associated with smaller LDL particle size (20.60 ± 0.05 nm versus 20.80 ± 0.03 nm in TT subjects) and also with larger VLDL size (47.6 ± 0.9 nm versus 46.5 ± 0.5 nm in TT subjects). Using the same statistical models in which fat intake was dichotomized according to its corresponding population mean, we did not uncover any significant interactions between the *APOA5* -1131T>C SNP and intake of total fat, SFAs, or MUFAs on concentrations of TGs, RLP-TGs, or RLP-C or on LDL and VLDL particle size.

To investigate whether the interaction between PUFA and *APOA5* -1131T>C was dose dependent, we analyzed, as continuous variables, PUFA intake as well as the intake of other fats to test the specificity of the effect. To

show the homogeneity by sex, separate models for men and women (Table 2) were fitted with interactions between fat intake (total fat, SFA, MUFA, and PUFA) and the *APOA5* polymorphism in determining TGs, RLP-TGs, and RLP-C concentrations and LDL and VLDL particle size by sex after controlling for potential confounders. Consistent with results for the categorical variables, we found significant interactions between PUFA intake and -1131T>C SNP on TGs, RLP-TGs, RLP-C, and VLDL size in men ($P=0.003$, $P<0.001$, $P=0.003$, and $P=0.002$, respectively) and in women ($P=0.001$, $P<0.001$, $P=0.005$, and $P=0.004$, respectively). Although the interaction for LDL particle size was statistically significant only in men ($P=0.004$), the direction of the effect was similar in women, and no significant heterogeneity by sex was found ($P=0.211$). In the combined analysis, all these interaction terms with PUFA intake were significant. An example of the modification of the effect of the SNP -1131T>C by PUFA intake on RLP-TG concentrations in men and women combined can be seen in Figure 2. Differences in slopes of the regression lines indicate that the effect of -1131T>C on RLP-TG depends on the amount of PUFA consumed. High PUFA intake was associated with lower RLP-TG concentrations in TT individuals, whereas high PUFA intake was associated with elevated RLP-TG concentrations in carriers of the C allele. To determine whether the interaction effect on RLPs was dependent on fasting TGs, we adjusted the regression models in Table 2 for fasting TGs. After this adjustment, the statistical significance of the interaction term between the -1131T>C polymorphism and PUFA intake in determining RLP decreased. For example, in the case of plasma RLP-TGs, the probability value for the interaction term for men and women (analyzed together) changed from $P<0.001$ (with no adjustment for fasting TGs) to $P=0.035$ (after adjustment for fasting TGs), revealing some degree of independence. However, considering the high correlation between fasting TGs and RLP-TGs ($r=0.8$, $P<0.001$ in the present study), we cannot conclude a clear independence of the effects. Finally, n-6 and n-3 FA intakes were taken as 2 categorical variables, according to the population mean (5.10% of energy for n-6 and 0.69% of energy for n-3). Because no heterogeneity of the effects by sex was detected, data for men and women were analyzed together. After adjustment for covariates (sex, age, familial relationships, BMI, smoking, alcohol use, total fat intake, carbohydrates, energy intake, diabetes status, menopausal status, β -blocker use, and use of estrogens), statistically significant interaction effects ($P=0.007$) were found for n-6 FA intake and the -1131T>C polymorphism in determining fasting TGs (113 ± 3 and 114 ± 3 mg/dL in TT subjects versus 120 ± 7 and 141 ± 7 mg/dL in carriers of the C allele when consuming less or more than 5.1% of energy from n-6) and RLP-TGs (Figure 3A). No statistically significant interactions were observed between the -1131T>C and n-3 PUFA on fasting TGs ($P=0.668$) or RLP-TGs (Figure 3B). Results for RLP-TC and VLDL and LDL particle size depending on the type of PUFA were similar. Thus, statistically significant interactions were

TABLE 2. Interaction Between Fat Consumption (as Continuous) and the -1131T>C Polymorphisms on TG-Rich Particle Concentrations and the Size of LDL and VLDL: Multiple Regression Analysis by the Type of Fat Consumed

	Total Fat				SFA				MUFA				PUFA			
	Men		Women		Men		Women		Men		Women		Men		Women	
	B	P	B	P	B	P	B	P	B	P	B	P	B	P	B	P
TG,* mg/dL																
CT+CC genotype	1.280	0.533	-1.502	0.217	1.742	0.078	1.098	0.627	1.227	0.573	-1.149	0.631	-1.532	0.031	-1.497	0.018
Fat intake	-1.003	0.955	1.002	0.639	-1.006	0.662	-1.003	0.827	1.033	0.161	-1.139	0.755	-1.002	0.863	-1.008	0.435
Interaction term	-1.003	0.530	1.019	0.091	-1.038	0.176	1.006	0.754	-1.005	0.872	1.027	0.308	1.105	0.003	1.096	0.001
RLP-TG,* mg/dL																
CT+CC genotype	-1.257	0.376	-1.855	0.209	2.148	0.055	1.076	0.789	-1.426	0.472	-1.166	0.707	-2.944	0.009	-2.038	0.002
Fat intake	-1.004	0.260	1.003	0.593	1.029	0.155	-1.017	0.369	-1.009	0.801	-1.013	0.663	-1.035	0.914	-1.002	0.902
Interaction term	1.011	0.942	1.028	0.105	-1.067	0.116	0.012	0.622	1.036	0.419	1.032	0.389	1.226	<0.001	1.161	<0.001
RLP-C,* mg/dL																
CT+CC genotype	1.014	0.575	-1.267	0.403	1.757	0.016	1.121	0.446	-1.115	0.681	1.001	0.998	-2.291	0.003	-1.439	0.016
Fat intake	-1.002	0.584	1.002	0.303	1.013	0.267	-1.014	0.129	1.011	0.549	-1.013	0.414	-1.023	0.029	-1.006	0.437
Interaction term	1.018	0.866	1.012	0.245	-1.047	0.022	-1.002	0.754	1.015	0.529	1.009	0.665	1.172	0.003	1.075	0.005
LDL particle size, nm																
CT+CC genotype	0.453	0.110	0.151	0.604	0.112	0.670	-0.079	0.653	0.409	0.193	0.034	0.886	0.519	0.012	0.116	0.396
Fat intake	0.004	0.352	-0.003	0.425	0.011	0.448	0.018	0.129	0.001	0.976	0.038	0.055	0.001	0.919	0.022	0.057
Interaction term	-0.017	0.069	-0.008	0.433	-0.024	0.299	0.001	0.974	-0.047	0.071	-0.001	0.641	-0.111	0.002	-0.032	0.135
VLDL particle size, nm																
CT+CC genotype	-10.880	0.843	-11.360	0.049	5.672	0.166	-2.117	0.499	-4.869	0.317	-9.277	0.036	-8.404	0.031	-8.907	0.008
Fat intake	0.048	0.512	-0.074	0.282	0.473	0.053	0.107	0.669	-0.041	0.929	-0.581	0.101	-0.443	0.071	-0.215	0.297
Interaction term	0.100	0.575	0.404	0.042	-0.538	0.136	0.253	0.404	0.412	0.328	0.885	0.029	1.409	0.004	1.553	0.004

Adjusted *P* values and regression coefficients (B, in milligrams per deciliter or nanometers, depending on the trait) for the main effect (genotype and fat) and interaction terms in men and women. Multivariate regression models for each type of fat and lipid trait were fitted. Models were adjusted for age, body mass index, familial relationships, smoking, alcohol, diabetes, estrogens, menopause, β -blockers, energy intake, carbohydrates, and total fat intake. The TT genotype was considered the reference category for the *APOA5* -1131T>C polymorphism.

Slopes for the TT and C carriers for each type of fat can be easily obtained: The corresponding fat intake term is the TT slope; the TC+CC genotype term \times the corresponding interaction term + the TT slope is the slope for carriers of the C allele for each lipid variable.

*For log-transformed variables, antilog values were calculated.

found for n-6 intake ($P=0.004$, $P=0.006$, and $P=0.01$, respectively), and nonsignificant interactions ($P>0.05$) for any parameter were detected in the case of n-3 PUFA. To illustrate the possible dose-response relationship in this interaction, n-6 and n-3 intake were considered as population tertiles (<4.37%, 4.37% to 5.54%, and >5.54% of energy for n-6 and <0.58%, 0.58% to 0.74%, and >0.74% of energy for n-3). We observed significant interactions ($P<0.01$) between -1131T>C and n-6 PUFA intake on the above variables with a clear dosage effect, whereas no significant interactions ($P>0.05$) between the -1131T>C polymorphism and n-3 PUFA were found. We present data obtained for fasting TGs and RLP-TGs as examples. Thus, a significant increase in TGs (+26%) and RLP-TGs (+38%) was observed when n-6 PUFA intake increased from the first to the third tertile in carriers of the -1131C allele (116 \pm 9, 124 \pm 9, and 146 \pm 8 mg/dL, respectively, for TGs and 17.6 \pm 2.9, 18.5 \pm 3.4, and 24.3 \pm 3.5 mg/dL, respectively, for RLP-TGs). However, no increase (0.1%, $P=0.952$) in TGs or even a nonsignificant decrease (-12%, $P=0.092$) in RLP-TGs was observed in TT homozygotes as n-6 PUFA increased. With regard to n-3 PUFA, no increase in TGs or in RLP-TGs in carriers of the -1131C allele was found. Moreover, in these subjects, a

decrease in RLP-TG was observed as PUFA intake increased. This decrease in RLP-TG concentrations did not reach statistical significance ($P>0.05$), but it was statistically significant in TT homozygotes (-23%, $P=0.005$). These results obtained with n-6 and n-3 PUFAs suggest that the effect observed for total PUFAs may be specific to the consumption of n-6 PUFAs.

Discussion

We found a significant and consistent interaction between the *APOA5* -1131T>C polymorphism (representing haplotype *APOA5**2)¹⁸ and PUFA intake on the concentration of plasma TGs, RLP-TGs, and RLP-C as well as on VLDL and LDL sizes in the Framingham Heart Study. This gene-diet interaction was found in related and in unrelated subjects and was not confounded by ethnicity. When PUFA consumption was >6% (population mean) of total energy intake, carriers of the -1131C allele exhibited significantly higher concentrations of fasting TGs and remnant lipoproteins (RLP-TGs and RLP-C). Moreover, compared with TT individuals, carriers of the -1131C allele displayed larger VLDL and smaller LDL molecules, which have been reported to increase CVD risk.³⁵ This gene-diet interaction showed a clear dose-dependent effect as well as a biologically plausible

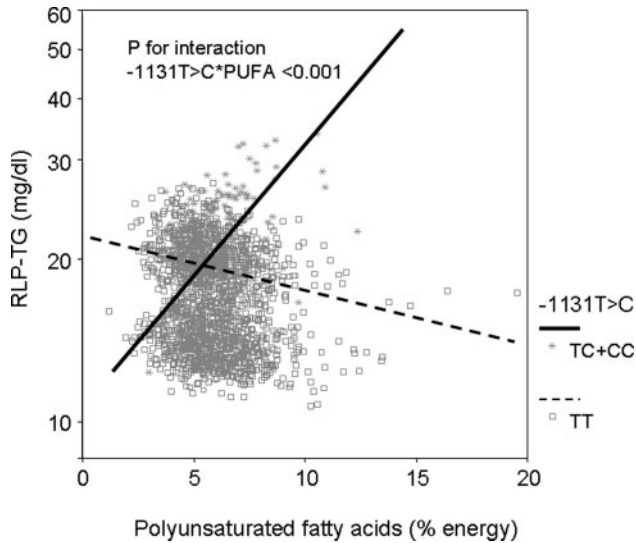


Figure 2. Scatterplot of the observed values (open squares indicate TT allele carriers; asterisks, C allele carriers) and regression lines of predicted values (broken lines indicate TT allele carriers; solid lines, C allele carriers) of RLP TG concentration by *APOA5* -1131T>C genotypes (in men and women combined, $n=1527$) depending on the PUFA consumed (as a continuous variable). Predicted values were calculated from the regression models containing PUFA intake, -1131T>C polymorphism, their interaction term, and the potential confounders (sex, age, familial relationships, BMI, smoking, alcohol, diabetes status, menopausal status, total fat intake, carbohydrates, estrogens, β -blocker use, and energy). R^2 of the model=0.14; $P<0.001$.

association consistent with the expected metabolic pathways involved.^{12,15,16} Two additional findings add interest to this observation. First, the interaction observed for SNP -1131T>C was not shared by SNP 56C>G, which represents the other common *APOA5**3 (56C>G) haplotype,¹⁸ despite the fact that we reported similar associations between these 2 haplotypes and plasma TRL concentrations.²² The contrasting results suggest that the hypertriglyceridemic phenotype associated with these 2 haplotypes is driven by different mechanisms. Thus, the causative mutation for the *APOA5**2 haplotype appears to be associated with dietary fat, whereas that of the *APOA5**3 haplotype may not be modulated by fat intake. Second, the reported interactions are exclusively due to consumption of PUFA, with no interactions detected for total fat, SFA, or MUFA, adding evidence to the prominent role of PUFAs as modulators of genetic effects in lipid metabolism.²⁵ Although it has been proposed that postprandial TRLs could be independent CVD risk factors and although the hypothesis is physiologically sound,³⁶ we found significant gene-PUFA intake interaction for both fasting TG and RLP concentrations. Given the fact that fasting TGs are highly correlated with RLP-TGs, we cannot conclude in the present study a clear independence of the effects on both variables. This is consistent with recent findings that have failed to support such independence at the population level.^{37,38} Therefore, it will be important from a scientific point of view to clarify the role of remnants in atherogenesis by using appropriate experimental designs; however, from a clinical point of view, the measurement of

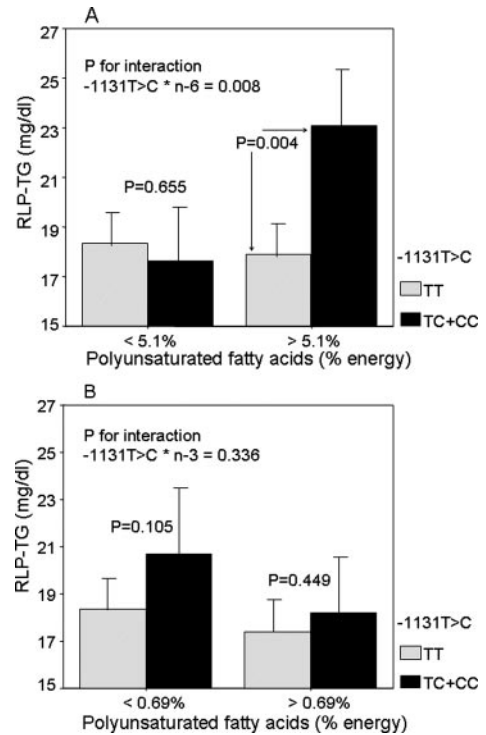


Figure 3. Mean RLP TG concentration in men and women combined depending on n-6 PUFA (A) and n-3 PUFA (B) categories (below and above the population mean) and *APOA5* -1131T>C genotypes (TT or -1131C carriers). Means were adjusted for sex, age, familial relationships, BMI, smoking, alcohol, diabetes status, menopausal status, estrogens, β -blocker use, total fat intake, carbohydrates, and energy. Mean values are antilog of the log value. Bars indicate standard error (SE) of means. SEs were computed to maintain the proportion estimated with the log transformation.

total TGs appears to be a reasonable and practical surrogate to identify disturbances in TRL metabolism and gene-diet interactions.

Because n-3 and n-6 FAs differ in their potential preventive effect on CVD,³⁹ we further investigated whether the PUFA interaction applied to the consumption of both families of PUFAs. Our results support the notion that the above-reported interactions are specific to n-6 PUFA. The potentially negative effects associated with elevated lipoprotein remnant concentrations observed in carriers of the *APOA5* -1131C allele who consume high n-6 PUFA were not observed for the consumption of n-3 PUFA. In fact, dietary n-3 FAs decreased RLP and related variables regardless of genotype. Similar findings have been reported for the arachidonate 5-lipoxygenase gene. Promoter variants at this locus are associated with increased atherosclerosis, and this association is promoted by dietary n-6 PUFA and inhibited by marine n-3 PUFAs.⁴⁰

The relevance of these findings for CVD risk detection and prevention lies in the correlation between fasting and postprandial TG metabolism and CVD risk.^{3,41} Fruchart-Najib et al¹¹ reported that overexpression of human *APOA5* diminished postprandial TG response to sunflower

oil in mice. Therefore, the observed differences between carriers of different alleles at *APOA5* -1131T>C, although still controversial,⁴² may be due to their differential expression. On the basis of the correlation between fasting and postprandial TG concentrations, we anticipate that compared with TT homozygotes, the *APOA5* -1131C minor allele will result in higher postprandial lipemia. In this regard, Jang et al²⁴ demonstrated that carriers of the -1131C allele had a higher chylomicron-TG area under the curve than did homozygotes for the -1131T allele after a fat load enriched in n-6 PUFA. Conversely, the allelic differences were not significant when subjects were provided with a low fat load.²⁴ Martin et al²³ also reported that male carriers of the -1131C allele had significantly higher lipemic responses to a fat tolerance test. However, the responses were not significantly different by genotype after adjustment for fasting TG concentrations. Moreover, Masana et al,⁴³ reported that carriers of the C allele had similar or even lower incremental diurnal triglyceridemia than did wild-type carriers after correction for fasting TG concentrations. We speculate that the high saturated fat meal used by Dwyer et al⁴⁰ prevented the manifestation of the increased postprandial load, which appears to be preferentially driven by n-6 PUFA.

There is no proven mechanistic explanation for our differential findings for n-6 and n-3 FAs. However, on the basis of the evidence that TRLs are hydrolyzed by the lipolytic action of dimeric LPL, which is bound to heparan sulfate proteoglycans on the vascular endothelium, one can speculate that *APOA5* targets VLDL to proteoglycans, placing VLDL in proximity to LPL.^{14–17} Moreover, *APOA5* may activate proteoglycan-bound LPL by stabilizing the dimerized conformation or by binding to an LPL allosteric site. An important feature of this model is that for all this to happen efficiently, LPL and *APOA5* must be in contact with proteoglycans on the endothelial cell membrane.¹⁷ In other words, there is margination or sequestration away from the blood of TRL during binding to endothelial LPL. The concept of margination has been illustrated by Park et al.⁴⁴ Most interesting is the fact that the amount of sequestration is dependent on the type of dietary fat. A diet rich in n-3 FAs promotes the margination of the TRLs, whereas a diet rich in n-6 FAs does not promote, and may even inhibit, the margination of TRLs. Therefore, we propose the following: (1) In subjects with the most common *APOA5* genotype, there is normal *APOA5*-induced margination of TRLs and normal hydrolysis of these lipoproteins. (2) In subjects carrying the -1131C allele, there is less *APOA5* available and less margination of TRLs, with subsequently increased levels of TRL, which may be accentuated in particles enriched in n-6 FAs.⁴⁵ (3) Increased presence of n-3 FA strongly enhances TRL margination by yet-unknown mechanisms but probably independent of *APOA5* levels. These effects overwhelm those milder effects associated with the *APOA5* variant.

These findings could be relevant within the context of future development of personalized dietary recommendations because the frequency of the -1131C allele varies

across ethnic groups, ranging from 0.07 in whites to 0.20 in Africans, 0.30 in Hispanics, and up to 0.40 to 0.50 in Chinese and Japanese populations.^{9,21,22} It is interesting to note that most dietary intervention studies using PUFA-rich diets have been performed in non-Hispanic whites,⁴⁶ in whom frequencies of the high-TG-associated *APOA5* alleles are relatively low. Hence, the overall CVD risk associated with the -1131C allele in the presence of high n-6 PUFA diets may not be apparent for the population as a whole.

In summary, the observed interaction between *APOA5* and PUFA consumption on TGs, RLP concentrations, and lipoprotein particle sizes suggests that higher n-6 PUFA intake increases plasma TGs and RLPs and decreases LDL particle size in carriers of the -1131C allele, contributing to a more atherogenic lipid profile for this subset of the population. Most interesting, this interaction was not observed for n-3 PUFA.

Medical societies and government bodies have embraced the concept of nutrition as a major player in the epidemic of cardiovascular disease (CVD) and potentially in its control. However, some scientists remain uncertain about what constitutes the optimal diet for atherosclerosis prevention and therapy. One of the major reasons for this uncertainty is the lack of conclusive results from dietary clinical trials. The bases for the inconsistencies are the many other factors affecting CVD risk and risk factors, including age, sex, physical activity, alcohol use, smoking, and genetic background. A more complete understanding of these factors and a thoughtful use of this information should help in the identification of vulnerable populations or persons who will benefit from more personalized and mechanistic-based dietary recommendations. This potential for better prevention and therapy can be developed within the context of nutritional genomics that may provide the tools to achieve effective dietary prevention and therapy for CVDs. In summary, some time in the future, clinicians will be able to quickly identify a patient's DNA profile for coronary artery disease risk and then get immediate information on his or her metabolic response to a particular diet. The findings of this research contribute to this future by showing that high dietary PUFA n-6 may not result in atherosclerosis protection for a subpopulation of subjects characterized by the presence of the polymorphism in the promoter region of the *APOA5* gene. However, this subgroup may substantially benefit from increased PUFA n-3 consumption.

Acknowledgments

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Disclosures

None.

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CLINICAL PERSPECTIVE

Medical societies and government bodies have embraced the concept of nutrition as a major player in the epidemic of cardiovascular disease (CVD) and potentially in its control. However, some scientists remain uncertain about what constitutes the optimal diet for atherosclerosis prevention and therapy. One of the major reasons for this uncertainty is the lack of conclusive results from dietary clinical trials. The bases for the inconsistencies are the many other factors affecting CVD risk and risk factors, including age, sex, physical activity, alcohol use, smoking, and genetic background. A more complete understanding of these factors and a thoughtful use of this information should help in the identification of vulnerable populations or persons who will benefit from more personalized and mechanistic-based dietary recommendations. This potential for better prevention and therapy can be developed within the context of nutritional genomics that may provide the tools to achieve effective dietary prevention and therapy for CVDs. In summary, some time in the future, clinicians will be able to quickly identify a patient's DNA profile for coronary artery disease risk and then obtain immediate information on his or her metabolic response to a particular diet. The findings of this research contribute to this future by showing that high dietary PUFA n-6 may not result in atherosclerosis protection for a subpopulation of subjects characterized by the presence of the polymorphism in the promoter region of the *APOA5* gene. However, this subgroup may substantially benefit from increased PUFA n-3 consumption.