Polyunsaturated Fatty Acids Interact with the PPARα-L162V Polymorphism to Affect Plasma Triglyceride and Apolipoprotein C-III Concentrations in the Framingham Heart Study

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ABSTRACT Peroxisome proliferator-activated receptor α (PPARα) is a nuclear transcription factor regulating multiple genes involved in lipid metabolism. It was shown that a common leucine to valine (L162V) substitution at the PPARα gene (PPARA) is functional and affects transactivation activity of PPARα ligands, such as PUFA, on a concentration-dependent basis. The current study examined this gene-nutrient interaction in relation to plasma lipid variables in a population-based study consisting of 1003 men and 1103 women participating in the Framingham cohort and consuming their habitual diets. We found significant gene-nutrient interactions between the L162V polymorphism and total PUFA intake, which modulated plasma triglycerides (TG; P < 0.05) and apolipoprotein C-III (apoC-III; P < 0.05) concentrations. The 162V allele was associated with greater TG and apoC-III concentrations only in subjects consuming a low-PUFA diet (below the population mean, 6% of energy). However, when PUFA intake was high, carriers of the 162V allele had lower apoC-III concentrations. This interaction was significant even when PUFA intake was considered as a continuous variable (P = 0.031 for TG and P < 0.001 for apoC-III), suggesting a strong dose-response effect. When PUFA intake was <4%, 162V allele carriers had ~28% higher plasma TG than did 162L homozygotes (P < 0.01). Conversely, when PUFA intake was >8%, plasma TG in 162V allele carriers was 4% lower than in 162L homozygotes. Similar results were obtained for (n-6) and (n-3) fatty acids. Our data show that the effect of the L162V polymorphism on plasma TG and apoC-III concentrations depends on the dietary PUFA, with a high intake triggering lower TG in carriers of the 162V allele. J. Nutr. 135: 397–403, 2005.

KEY WORDS: • PUFA • PPARα, gene-nutrient interaction • triglycerides

PUFA, one of the dietary components that appear to have major importance in modulating gene expression, are currently the focus of special interest in vitro studiess in nutrigenomics (1,2). Regulation of gene expression by PUFA can occur through interaction with specific or nonspecific ligands that bind to response factors acting on cis-regulatory elements of the gene, which turn on or off mRNA synthesis (3). Specifically, it was demonstrated that PUFA can interact directly with transcription factors such as peroxisome proliferator-activated receptor α (PPARα), a nuclear transcription factor that regulates multiple genes involved in lipid homeostasis, particularly the metabolism of triglyceride-rich lipoproteins (TRLs) and HDL (4,5). Several common genetic variants in the PPARα gene (PPARA) have been described (6–9). One of these polymorphisms, consisting of a G 484 C transversion at the first base of codon 162, creates a missense mutation that alters leucine to valine (L162V) and has functional consequences on receptor activity (6,7). Interestingly, it was shown that the effect of the L162V polymorphism on the transcriptional activation associated with ligand binding to PPARα depends on the concentration of the ligand to which it is exposed (4,5). A wide range of compounds were identified as natural or synthetic ligands for PPARα. Fatty acids, mainly PUFA and PUFA-derived compounds, are natural ligands of PPARα, whereas fibrate are synthetic agonists of PPARα (5,10). It was reported that at high concentrations of ligands,
ligand binding is associated with increased transcriptional activation by the 162V allele compared with the 162L allele. However, at low concentrations, the situation is reversed, i.e., the 162V allele is associated with reduced transcriptional activation (7,8). On the basis of these findings, we hypothesized that dietary PUFA can act as natural ligands for PPARα, giving rise to gene-diet interactions at the population level that may mask the effect of the PPARA-L162V polymorphism on plasma lipid concentrations if these interactions are not considered. In a previous study, we examined associations between the L162V polymorphism and plasma lipids in the Framingham Offspring Study (11). We found that the presence of the 162V allele was associated with higher plasma concentrations of total and LDL cholesterol (LDL-C). Despite the prominent role that PPARα plays in the regulation of proteins involved in TRL and HDL metabolism, no association was found between plasma triglyceride (TG) or HDL-C concentrations and the presence of the 162V allele. To test the hypothesis involving the PUFAs-PPARA interaction at the population level, we studied the potential modification of the effect of the PPARA-L162V polymorphism on plasma lipid, lipoprotein, and apolipoprotein concentrations relative to the habitual dietary PUFA intake in the Framingham Offspring Study. In addition, given the reported differential effects of dietary (n-3) and (n-6) PUFA on plasma lipid concentrations, inflammatory response, and cardiovascular risk (12), we also examined the specific effect of (n-3) and (n-6) PUFA on this gene-diet interaction.

SUBJECTS AND METHODS

Subjects and study design. The study sample consisted of 1003 men and 1103 women who participated in the Framingham Offspring Study. Detailed design and methodology for the Framingham Offspring Study were described previously (13,14). The analyses carried out in this work included data collected from the 5th examination visit of the study carried out in the years 1992–1995. The Institutional Review Board for Human Research at Boston University and the Human Investigation Research Committee at Tufts University/New England Medical Center approved the protocol. All participants provided written informed consent, underwent standardized clinical examination, and provided fasting blood samples. PPARA genotypes, clinical, biochemical and dietary data were determined. Subjects with complete data for the variables under study were included in this analysis, with the exception that those taking lipid-lowering medications (n = 166) were excluded.

Alcohol consumption was calculated in g/wk based on the reported alcoholic beverages consumed in the previous year for each individual, and subjects were classified as nondrinkers (those who did not report consumption of alcohol), and drinkers (13,14). Smokers were defined as those who smoked at least 1 cigarette/d. Diabetes mellitus was classified according to the criteria recommended by the American Diabetes Association (15).

Biochemical determinations. Venous blood samples were collected from fasting subjects and plasma was separated from blood cells by centrifugation at 1100 × g for 10 min and used immediately for the measurement of lipids. Plasma lipids, lipoproteins, and apolipoproteins were measured as previously described (14,16). Lipoprotein subclass distributions were determined by proton NMR spectroscopy as previously described (14,17). Each profile displayed the concentrations of 6 VLDL, 1 intermediate density lipoprotein, 3 LDL, and 5 HDL subclasses and the weighted-mean particle sizes of VLDL, LDL, and HDL. The 9 lipoprotein subclass categories used were the following: large VLDL (80–220 nm), intermediate VLDL (35–80 nm), small VLDL (27–35 nm), large LDL (21.3–27.0 nm), intermediate LDL (19.8–21.2), small LDL (18.3–19.7 nm), large HDL (8.8–13.0 nm), intermediate HDL (7.8–8.8 nm), and small HDL (7.3–7.7 nm).

Dietary information. Dietary intake was estimated with the semiquantitative FFQ described and validated by Rimm et al. (18). This questionnaire includes 136 food items with specified serving size. Subjects were asked to report their frequency of use of each item per day, week, or month over the past year by checking 1 of the 9 frequency categories. Food-item intake frequencies are linked with nutrient data at Harvard University to estimate daily nutrient intakes and energy. The mean daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items. The Harvard University Food Composition Database derived from USDA sources and supplemented with manufacturer information was used to calculate nutrients. This FFQ was used successfully by numerous studies and was validated extensively for a variety of nutrients including total PUFA intake and (n-3) or (n-6) fatty acids (18,19). Fat intake data were first calculated in absolute amounts (g/d). We then modeled the effect of fat in terms of nutrient density, i.e., the ratio of energy from fat to total energy, expressed as a percentage. Intakes of total fat, SFA, monounsaturated fatty acids (MUFA), total PUFA, (n-3) fatty acids, and (n-6) fatty acids 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 by mean value of the population (intakes below vs. above the mean). In addition, we defined 3 categories of PUFA intake (low, <4% of energy; medium, 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. We considered together, as (n-3) fatty acids, the dietary intake of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid. Linoleic acid (LA) and arachidonic acid (AA) were grouped as (n-6) fatty acids.

Genetic analyses. Genomic DNA was isolated from peripheral blood leukocytes by standard methods. Genotyping was carried out on a Perkin Elmer/Applied Biosystems 7700 sequence detection system (Perkin Elmer) using Taqman® probes for allelic discrimination as previously described (11).

Statistical analyses. We examined all continuous variables for normality of distribution. TG concentrations were log transformed. The relation between PPARA genotypes, dietary PUFA, and lipid or apolipoprotein measures was evaluated by analysis of covariance techniques. Because our study involved correlated data due to familial relations (siblings and cousins), we employed a generalized linear mixed model approach to adjust for this correlation. We assumed exchangeable correlation structure and performed the analysis using the Mixed procedure in SAS.

The interaction between dietary PUFA (as a continuous or as a categorical variable) and the PPARA polymorphism was tested in a hierarchical multivariate interaction model after controlling for potential confounders including gender, age, BMI, smoking, alcohol consumption, diabetes status, β-blocker use, diuretic use, estrogen use (in women), energy intake, and the percentage of energy intake from total fat. These analyses were performed for the whole population and for men and women separately to check the homogeneity and the magnitude of the effect. Predicted values of selected variables [TG and apolipoprotein (apo)C-III] were computed and displayed against the percentage of daily energy intake from PUFA and the PPARA-L162V polymorphism. Standard regression diagnostic procedures were used to ensure the appropriateness of these models. When PUFA intake was considered to be a continuous variable, its interaction with the PPARA 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 PPARA genotype. All reported probability tests were two-sided. Statistical analysis was carried out using SAS software.

RESULTS

The interaction between dietary PUFA intake and the PPARA-L162V polymorphism was analyzed in 1003 male and 1103 female (Table 1) participants in the Framingham Study who had genetic, biochemical, and dietary data. The mean intake of dietary PUFA for the whole population was ~6% of dietary energy intake. The main source of PUFA was vegetable
Effect of dietary PUFA intake on triglyceride (TG) concentrations in carriers of the 162L allele, PUFA intake did not decrease either TG or apoC-III concentrations. In contrast, among homozygotes for the 162V allele, PUFA intake did not decrease either TG or apoC-III concentrations. Moreover, we examined the effect of PUFA intake as a continuous variable to avoid the problem of selection of cutoff points. In agreement with the data obtained using dietary PUFA as a categorical variable, the modification of the effect of the L162V polymorphism by PUFA intake seemed to be linear in determining plasma TG (Fig. 1A) and apoC-III (Fig. 1B) concentrations. Significant interaction terms between PUFA intake as a continuous variable and the PPARα polymorphism were found for both TG (P = 0.031) and apoC-III (P < 0.001) concentrations after adjustment for sex, age, BMI, tobacco smoking, alcohol consumption, diabetes, β-blockers, diuretics, estrogen use (in women), energy intake, and the percentage of energy intake from total fat.

Finally, the specific effect for (n-6) and (n-3) fatty acid fatty acids on this gene-diet interaction was explored. Thus, we first examined (n-6) and (n-3) fatty acid fatty acids as 2-category variables, using the population mean (5.10% of energy for (n-6) and 0.69% of energy for (n-3)). After adjustment for covariates, significant interaction terms were found for (n-6) fatty acid fatty acids intake in determining plasma TG (Fig. 2A) and apoC-III (Fig. 2B) concentrations. Similar gene-diet interaction effects on plasma TG (Fig. 3A) and apoC-III concentrations (Fig. 3B) were found for dietary (n-3) fatty acids. However, only the effect on apoC-III concentrations was significant due to the additional decrease in TG concentrations in 162L homozygotes who consumed more (n-3) fatty acids than the population mean. Overall, this gene-diet interaction involving the PPARα L162V polymorphism and dietary PUFA intake shows that carriers of the 162V allele had significantly lower plasma TG and apoC-III concentrations when consuming a high PUFA diet, in which (n-6) and (n-3) fatty acids seem to have a similar role.
concentrations of PPAR activating a dose-response relation. In addition, it was reported that activation increases TRL catabolism by downregulation of apoC-III, a lipoprotein lipase inhibitor (20) that in- volves in the regulation of the 162V allele at high concentrations. These results agree with previous evidence modulating the effects of the this polymorphism on lipid metabolism in humans. These results agree with previous evidence 

**TABLE 2**

| Lipid and lipoprotein concentrations according to the level of PUFA intake and the PPARA-L162V polymorphism in men and women |
|----------------------------------|----------|----------|----------|----------|----------|----------|
|                                | PUFA < 4% | PUFA 4–8% | PUFA > 8% | P-value3 |
| Total cholesterol,4 mg/dL       | 202 ± 3   | 215 ± 6  | 203 ± 1   | 210 ± 2  | <0.01    | 198 ± 3   | 215 ± 7  | 0.03  | 0.33 |
| LDL-C,4 mg/dL                   | 125 ± 3   | 134 ± 6  | 125 ± 1   | 133 ± 2  | <0.01    | 119 ± 4   | 135 ± 7  | 0.02  | 0.486 |
| HDL-C,4 mg/dL                   | 51 ± 1    | 50 ± 2   | 50 ± 1    | 50 ± 1   | 0.74     | 51 ± 1    | 52 ± 3   | 0.64  | 0.772 |
| Triglycerides,5 mg/dL           | 117 ± 1   | 150 ± 1  | 121 ± 1   | 122 ± 1  | 0.84     | 124 ± 1   | 119 ± 1  | 0.74  | 0.048 |
| Apo B,5 mg/dL                   | 109 ± 2   | 126 ± 5  | 111 ± 1   | 115 ± 2  | 0.02     | 108 ± 2   | 115 ± 6  | 0.31  | 0.113 |
| Apo C-III,5 mg/dL               | 16.3 ± 0.4| 19.7 ± 1  | 16.1 ± 0.1| 16.7 ± 0.4| 0.12     | 16.9 ± 0.4| 15.4 ± 0.9| <0.01 | 0.03 |
| Apo A-I,6 mg/dL                 | 155 ± 2   | 156 ± 5  | 153 ± 1   | 152 ± 2  | 0.6      | 152 ± 2   | 155 ± 5  | 0.58  | 0.746 |

1 Values are means ± SEM. Values were adjusted for gender, age, familial relationships, BMI, smoking, alcohol consumption, and energy and total fat intake. PUFAs intake is expressed as a percentage of total energy.

2 P-value obtained in the comparison of means between PPARA genotypes (carriers of the 162V allele vs. 162L homozygotes) according to the PUFAs intake.

3 P-value obtained for the interaction term between the PPARA-L162V polymorphism and PUFA intake in the multivariate regression model adjusted for the covariates.

4 1 mg/dL = 0.0259 mmol/L.

5 1 mg/dL = 0.0113 mmol/L.

6 1 mg/dL = 0.01 g/L.

**DISCUSSION**

We found a significant interaction between the PPARA-L162V polymorphism and PUFA intake on plasma TG and apoC-III concentrations in a large sample of free-living individuals, suggesting that habitual dietary PUFA intake can modulate the effects of the this polymorphism on lipid metabolism in humans. These results agree with previous evidence from experimental studies involving this gene in which ligand binding was associated with increased transcriptional activation by the 162V allele compared with the 162L allele at high concentrations of PPARα ligands such as fatty acids (7,8), thus adding evidence to this observation. Accordingly, based on our knowledge regarding the function of the 162V variant of PPARα, we hypothesized that at low intakes of dietary PUFA (<4%), activation of PPARα would be reduced resulting in higher TG concentrations in carriers of the 162V allele, whereas at high levels of PUFA intake (≥8%), lower plasma TG concentrations would result from increased activation of PPARα. Our results are consistent with this hypothesis, showing a dose-response relation. In addition, it was reported that PPARα activation increases TRL catabolism by downregulation of apoC-III, a lipoprotein lipase inhibitor (20) that inhibits lipolysis of TRLs by lipoprotein lipase, thus decreasing plasma TG (21). Our hypothesis is further supported by the finding that the association between plasma apoCIII and the L162V polymorphism mirrors that of plasma TG. No PPARA-PUFA interaction in determining total cholesterol, LDL-C or apoB concentrations was found in our study. This means that the effects of the PPARA-L162V polymorphism on total cholesterol, LDL-C, and apoB concentrations do not vary depending on the PUFA intake. Therefore, the lack of modulation of these variables by dietary intake may explain the greater consistency of different studies analyzing the effect of the PPARA-L162V polymorphism on plasma lipid concentrations. For example, Vohl et al. (6) and Robitalle et al. (22), as well as of our previous results in the Framingham population (11) showed that carriers of the 162V allele had higher plasma LDL-C and apoB concentrations. In the present work, the effect of the PPARA-L162V polymorphism on these variables did not differ across the categories of PUFA intake. To date, only 1 study has reported on the modulation by dietary fat of the effect of the PPARA-L162V polymorphism on plasma lipid concentrations. This study was conducted by Robitalle et al. (22) in a sample of men with and without the metabolic syndrome and found no interaction with total or saturated fat in determining total cholesterol, LDL-C, HDL-C, TG, or apoB concentrations. Conversely, they found a significant interaction between the L162V polymorphism and total fat intake or saturated fat intake in determining waist circumference, and concluded that waist circumference increased with a higher intake of dietary fat in 162L homozygotes. However, waist circumference was not influenced by dietary fat intake among carriers of the 162V allele. Unfortunately, they did not analyze the potential interaction between this polymorphism and PUFA intake on plasma lipids; thus, we cannot compare our results. Moreover, they did not report the mean intake of dietary PUFA in the population, data that would have been very useful to test our hypothesis because that they found...
significant differences in plasma TG concentrations between the 2 genotype groups, i.e., carriers of the 162V allele had higher concentrations than 162L homozygotes.

On the other hand, Flavell et al. (6), in a study of 121 diabetic subjects, observed a trend toward lower TG concentrations in carriers of the 162V allele, pointing out that this was not significant due to high individual variability of plasma TG concentrations and low sample size. However, they found a relevant and significant increase in apoA-I concentrations in subjects with diabetes who carried the 162V allele.

PPARα regulates the major apolipoprotein components of HDL (apoA-I and apoA-II), we did not find an association between apoA-I or HDL-C concentration and the PPARα polymorphism in the Framingham Study (11). In the present work, no significant interaction between PUFA intake and this polymorphism in determining HDL-C or apoA-I concentrations was observed. However, in experimental studies,
PPARα activation also leads to qualitative modification of HDL. In addition, fibrate treatment of mice expressing human apoA-1 activates PPARα, induces phospholipid transfer protein (PLTP), and results in the appearance of large cholesterol ester–enriched HDL particles (23). In agreement with these observations and consistent with our previous results, we found that the interaction term between PUFA intake and the PPARA-L162V polymorphism was of borderline significance (\( P = 0.062 \)) in predicting the concentration of small HDL. We hypothesize that in carriers of the 162V allele, the high levels of small HDL with low PUFA intake reflect decreased transcriptional activation of PLTP by the mutant receptor. In contrast, with high PUFA intake, increased transcriptional activation of PLTP results in larger HDL particles and lower levels of small HDL.

When analyzing the specific effect of both (n-3) and (n-6) fatty acids on the interaction with the PPARA-L162V polymorphism, we found similar results in terms of their influence on decreasing plasma TG and apoC-III concentrations in carriers of the 162V allele. Although little is known about the differential effect of (n-3) and (n-6) fatty acids on the ligand-binding activity of PPARA and specific transactivation in experimental studies (24–26), it is thought that both (n-3) and (n-6) can regulate the activity of PPARα (27). Moreover, some studies showed that although (n-3) are more potent than (n-6) as in vivo activators of PPARα (26,28), neither family of PUFAs is a particularly strong PPARα activator compared with PUFAs metabolites such as eicosanoids or oxidized fatty acids that have 1 or 2 orders of magnitude greater affinity for PPARα (29). In analyzing the specific interaction of these families of PUFAs, we discovered that the interaction between (n-3) fatty acids and the L162V polymorphism was not significant in determining plasma TGs, whereas that for (n-6) fatty acids was significant. The reason for this lack of significance is that both (n-6) and (n-3) fatty acids decrease TG concentrations in carriers of the 162V allele. In addition, a higher intake of (n-3) fatty acids also decreased TG concentrations in L162 homozygotes. The hypotriglyceremic effect of the high consumption of (n-3) fatty acids (mainly EPA and DHA) was reported consistently in experimental and observational studies (30–33). However, although LA is the most potent dietary fatty acid for reducing plasma total-C and LDL-C (34,35), no hypotriglyceremic effect of (n-6) fatty acids is consistently recognized (12). Our study shows that the reduction in TGs associated with higher intakes of (n-6) fatty acids is limited to carriers of the 162V allele. Considering that the prevalence of this group is small in the population (14%), this effect is clearly masked when the population as a whole is analyzed.

In summary, the gene-nutrient interaction between the PPARA-L162V polymorphism and PUFA intake observed at the population level is in agreement with the results from experimental studies involving the expression of the PPARA because it is consistent with the reported function for this gene. This interaction may help to explain conflicting results regarding the influence of the PPARA-L162V polymorphism on plasma TG and apoC-III concentrations, as well as on the controversial suggestion of cardiovascular risk that was reported for this polymorphism. Moreover, this interaction may help to explain the intradividual differences in the plasma TGs in response to the PUFA content of the diet and even to distinguish the hypotriglyceremic effect of (n-6) and (n-3) fatty acids. Thus, (n-6) fatty acids may specifically decrease plasma TG concentrations in carriers of the 162L allele, whereas dietary (n-3) fatty acids may exert a hypotriglyceridemia effect independently of the PPARA-L162V polymorphism. However, this point requires further investigation using appropriate intervention studies.

LITERATURE CITED


