APOA5 Gene Variation Interacts with Dietary Fat Intake to Modulate Obesity and Circulating Triglycerides in a Mediterranean Population1,2

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Abstract

APOA5 is one of the strongest regulators of plasma TG concentrations; nevertheless, its mechanisms of action are poorly characterized. Genetic variability at the APOA5 locus has also been associated with increased cardiovascular disease risk; however, this predisposition could be attenuated in the context of a prudent diet as traditionally consumed in the Mediterranean countries. We have investigated the interaction between a single nucleotide polymorphism (SNP) at the APOA5 gene (-1131T > C) and dietary fat that may modulate TG-rich lipoprotein concentrations and anthropometric measures in overweight and obese participants. We recruited 1465 participants from a Spanish population (20–65 y old; BMI 25–40 kg/m2) attending outpatient obesity clinics. Consistent with previous reports, we found an association between the APOA5-1131T > C SNP and TG-rich lipoprotein concentrations that were higher in carriers of the minor allele than in noncarriers (P < 0.001). Moreover, we found a significant genotype-dietary fat interaction for obesity traits. Participants homozygous for the −1131T major allele had a positive association between fat intake and obesity, whereas in those carrying the APOA5−1131C minor allele, higher fat intakes were not associated with higher BMI. Likewise, we found genotype-dietary fat interactions for TG-rich lipoproteins (P < 0.001). In conclusion, we have replicated previous gene-diet interactions between APOA5−1131T > C SNP and fat intake for obesity traits and detected a novel interaction for TG-rich lipoprotein concentrations. Our data support the hypothesis that the minor C-allele may protect those consuming a high-fat diet from obesity and elevated concentrations of TG-rich lipoproteins. J. Nutr. 141: 380–385, 2011.

Introduction

Hypertriglyceridemia (HTG)6 has been identified as an independent risk factor for coronary heart disease (1–4). Moreover, HTG is a common feature of metabolic syndrome (MetS). Plasma TG concentrations are regulated by complex pathways involving many factors, among them, lipoprotein lipase (LPL), and Apolipoprotein A5 (APOA5), C2, C3, and E, APOA5 being the least well characterized in terms of functionality. Studies performed using experimental models have shown that APOA5 overexpression in mice, either as a transgene or by adenovirus transfection, results in HTG (5).

The relevance of APOA5 to human TG-rich lipoprotein metabolism comes primarily from genetic studies, which have consistently shown associations between the APOA5 locus and plasma TG concentrations. Therefore, APOA5 has been established as a major genetic contributor to TG concentrations in the general population (6). Moreover, we have shown synergistic associations in combination with other candidate genes (7). However, inter-study variation in the strength of the reported associations suggests that additional gene-environment interactions may modulate those associations (8–13). In fact, evidence for APOA5-diet interactions has been previously demonstrated for plasma lipoprotein metabolism as well as BMI (14–20).

The traditional Mediterranean Diet, characterized by the relatively high consumption of MUFA from olive oil (21), has beneficial effects on cardiovascular risk factors such as hypertension, diabetes, obesity, and hypercholesterolemia (22). It has been proposed that our metabolism has adapted to function optimally within our long-term, traditional environment (23). Therefore, our ancestral metabolism may have adapted to a diet

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4 Abbreviations used: APOA5, apo A5; CVD risk, cardiovascular risk; MetS, metabolic syndrome; SNP, single nucleotide polymorphism.

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that is dramatically different from the saturated fat-rich and highly refined and processed foods currently consumed in Western societies (7). Therefore, it is possible to hypothesize that alleles that are associated with an increased disease risk today were silent in the presence of our traditional diet and lifestyle (24). To prove such a hypothesis, we investigated interactions between a common promoter single nucleotide polymorphism (SNP) at the APOA5 gene (-1131T > C) and dietary fat that may modulate the levels of TG-rich lipoproteins and anthropometric measures in a sample of overweight and obese white participants.

Materials and Methods

Participants and methods. We recruited overweight or obese participants (>25 kg/m² < BMI <40 kg/m²) within the age range of 20–65 y of age (n = 1465) who attended 5 outpatient obesity clinics between 2009 and 2010 in the city of Murcia, located in southeastern Spain. Patients receiving thernogenic or lipogenic drugs or those diagnosed with diabetes mellitus, chronic renal failure, hepatic diseases, or cancer were excluded from the study. All procedures were in accordance with good clinical practices. Written consent was obtained from each participant before participation and the study protocol was approved by the Research Ethics Committee of the Virgen de la Arrixaca Hospital. Participant identity remained confidential to guarantee anonymity.

Anthropometric and biochemical features. Participants were weighed while barefoot, wearing light clothes, on a digital scale that measured to the nearest 0.1 kg, at the same time of the day, once per week. Height was measured using a Harpenden digital stadiometer (height (m)/2. Total body fat was measured with bioelectrical impedance using TANITA TBF-300 (TANITA Corporation of America) equipment. Body fat distribution was assessed by anthropometric measures, including waist circumference, at the level of the umbilicus, and hip circumference, with the widest circumference over the greater trochanters (25). All measurements were made with a flexible and inextensible measuring tape.

Glucose, cholesterol (C), and TG concentrations of plasma and lipoproteins were determined by automated chemical analysis (II. ILAB 600 Chemistry Analyzer of Instrumentation Laboratory). VLDL were prepared by ultracentrifugation (26). HDL-C was measured after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium (27). LDL-C was calculated as TC minus HDL-C plus VLDL-C using the Friedewald equation, when the TG was <4.52 mmol/L (28). Insulin was determined through a solid-phase, 2-site chemiluminescent immunoassay (IMMULITE 2000 Insulin). Blood pressure was measured with participants seated with the arm resting on a table. Blood pressure readings were measured in mm Hg.

We used ATP III 2001 guidelines to classify patients for MetS, which was defined by the presence of 3 or more of the following characteristics: 1) central obesity: waist circumference ≥ 102 cm (men) or ≥ 88 cm (women); 2) HTG: TG ≥ 150 mg/dL (1.7 mmol/L); 3) low HDL-C: < 40 mg/dL (1.03 mmol/L) (men) or < 1.29 mmol/L (50 mg/dL) (women); 4) hypertension: blood pressure ≥ 130/85 mm Hg or taking medication; and 5) fasting plasma glucose ≥ 110 mg/dL (6.1 mmol/L) (29).

Habitual dietary intake. To evaluate food habits, initial nutrient intake was determined by a 24-h dietary recall. Interviews were conducted from Monday to Friday, including 24-h recalls of food intake from weekend and weekdays. Total energy intake and macro nutrient composition from the initial 24-h recalls were analyzed with the nutritional evaluation software program Grunurum (30) on the basis of Spanish food composition tables (31). The intakes of fatty acids were also calculated from Spanish food composition tables (32). The recorded intakes were typical of their usual diets. These calculations allowed us to estimate the fat quality percentage intakes from total grams of the major SFA, MUFA, and PUFA, including linoleic acid [18:2(n-6)], A-linolenic acid [18:3(n-6)], EPA [20:5(n-3)], and DHA [22:6(n-3)].

DNA isolation and genotyping. DNA was isolated from blood samples using routine DNA isolation sets (QiAGEN). We performed genotyping of APOA5 -1131T > C SNP using a TaqMan assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection system (Applied Biosystems) according to the standardized laboratory protocols (33). We selected this specific APOA5 SNP based on previous gene-diet interactions between APOA5 -1131T > C SNP and fat intake for obesity traits (14–20).

Statistical analyses. We used Pearson’s chi-square test and the Fisher test as statistical procedures to evaluate the distribution of the APOA5 gene SNP in our population. TG and VLDL concentrations were log transformed. We considered different genetic models (dominant and recessive) to determine the most appropriate model. We applied a dominant model in which carriers of 1 or 2 copies of the minor allele (11.8%; n = 153) were grouped and compared with major allele homozygotes (88.2%; n = 1143). We applied ANOVA and the Student’s t test to compare crude means across genotype groups. We performed multivariate adjustments of the associations by ANCOVA and estimated adjusted means. We adjusted analyses for potential confounders, including sex, age, nutrition center, smoking, and alcohol consumption. We fitted logistic regression models to estimate the OR and 95% CI of obesity and particular MetS components, including high TG, high glucose, high blood pressure, and abdominal obesity associated with the APOA5-1131T > C SNP. We also tested the statistical homogeneity of effects of sex in the corresponding regression model with interaction terms. Statistical analyses were performed using SPSS 15.0 software. A 2-tailed P-value < 0.05 was considered significant.

Results

The participants were in general sedentary and obese (Table 1); 23% were smokers and 73.4% reported regular consumption of alcoholic beverages. Their usual diets were characterized by lower percentages of carbohydrate and higher percentages of protein and fat than the current Spanish dietary recommendations (34). Nevertheless, over 50% of the energy supplied by fat was derived from MUFA, reflecting the traditional high consumption of olive oil. The distribution of the APOA5-1131T > C genotypes was consistent with Hardy-Weinberg equilibrium. TG and VLDL-C concentrations were significantly different among the -1131T > C variants and were higher in carriers of the minor allele than in noncarriers for TG (1.5 ± 0.61 vs. 1.1 ± 0.46 mmol/L (P = 0.02), respectively, and for VLDL (0.7 ± 0.32 vs. 0.6 ± 0.22 mmol/L (P = 0.01). No significant associations between gene variants with obesity or other MetS characteristics, such as waist circumference, fasting plasma glucose, systolic or diastolic blood pressure, fasting insulin, or HDL-C, were detected after adjusting for multiple testing.

APOA5 gene-diet interactions for obesity. To evaluate whether dietary intake modulated the association between -1131T > C SNP and dietary patterns, we first examined the effects of different macronutrients as categorical variables. For total fat, carbohydrate, and protein intakes, we classified the population into groups according to the median value of the population (91 g for fat, 200 g for carbohydrate, and 81 g for proteins).

We detected a gene-diet interaction between the -1131T > C SNP and fat intake (g) relative to BMI and waist and hip, and the same trend was found for body fat percent (Table 2) after controlling for sex, age, nutrition center, smoking, and alcohol consumption. Participants homozygous for the -1131T major
TABLE 1  Anthropometric, metabolic, and dietary characteristics of the population studied

<table>
<thead>
<tr>
<th>Age, y</th>
<th>39.4 ± 12.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric variables</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.1 ± 5.38</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.1 ± 17.3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.6 ± 0.08</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>37.3 ± 6.63</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>102.2 ± 15.07</td>
</tr>
<tr>
<td>MetS variables</td>
<td></td>
</tr>
<tr>
<td>Total C, mmol/L</td>
<td>2.2 ± 0.41</td>
</tr>
<tr>
<td>LDL-C, mmol/dL</td>
<td>1.3 ± 0.36</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.6 ± 0.18</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>0.2 ± 0.11</td>
</tr>
<tr>
<td>TG, mmol/dL</td>
<td>1.1 ± 0.60</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8 ± 0.91</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>59.1 ± 58.6</td>
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<tr>
<td>HOMA-IR</td>
<td>1.9 ± 2.2</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>11.6 ± 1.60</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>7.1 ± 1.1</td>
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</tbody>
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Dietary intake

| Total energy, kJ/day | 8650 ± 2990 |
| Proteins, % total energy | 17.0 ± 4.61 |
| Carbohydrates, % total energy | 41.8 ± 10.6 |
| Carbohydrates, g/d | 214.5 ± 88.7 |
| Fiber, g/d | 18.8 ± 11.1 |
| Fats, % total energy | 42.2 ± 9.61 |
| MUFA, % total fat | 55.5 ± 8.10 |
| PUFA, % total fat | 13.9 ± 4.01 |
| SFA, % total fat | 29.8 ± 8.54 |

Other characteristics

| Physical activity, MET | 4470 ± 7540 |
| Smokers, n (%) | 345 (23.3) |
| Obese, n (%) | 884 (53.8) |
| MetS, n (%) | 379 (23.0) |
| APOA5-1131T > C² | |
| CC, n (%) | 4 (0.3) |
| TC, n (%) | 149 (11.5) |
| TT, n (%) | 1143 (88.2) |

1 Values are means ± SD, n = 1465 (unless otherwise indicated) or percent.

To explore a possible dose-response relationship in the -1131T > C-fat interaction and to avoid the problem of selection cutoff points, we also considered total fat intake as a continuous variable. In agreement with the data obtained using fat intake as a categorical variable, there was an interaction between total fat intake and the -1131T > CC SNP for predicting TG plasma concentration (P = 0.003). We observed a similar interaction for VLDL (P = 0.002). For carriers of the minor allele (C), TG concentrations decreased as total fat increased (Fig. 2), whereas TG concentrations did not differ with fat intake in major allele homozygotes. Relationships between genotype and fat intake for TG were similar to those we detected for obesity-related traits. For both obesity and TG, increasing fat intake was associated with a beneficial metabolic outcome only in minor allele carriers. Of note, these significant interactions for TG were not eliminated by adjustment for obesity or by intake of other macronutrients.

Discussion

In this study performed in overweight and obese Spanish individuals, we replicated previous findings (15) obtained in a sample of the general North American population, supporting the notion that the associations and dietary interactions observed for the APOA5-1131T > C SNP and obesity could be generalized to a wide range of white men and women. Moreover, we obtained a significant gene-diet interaction between fat intake and the APOA5-1131T > C SNP for plasma TG concentrations. Interactions affecting 2 cardiovascular disease (CVD) risk factors (BMI and TG) could underlie the inconsistencies among reported associations between APOA5 variants and CVD risk (35).

In the multifactorial etiology of obesity, dietary fat, an energy-dense and highly palatable nutrient, has long been considered a key determinant of obesity (36). Moreover, its storage in the adipose tissue has been the preferred evolutionary mechanism for maintaining the energy reserves of the organism (37). However, intervention studies have shown that simply reducing dietary fat does not seem to be sufficient to accomplish successful and lasting weight reductions. Moreover, despite decreases in the average consumption of fat at the population level, obesity continues to rise (38,39). One obvious explanation for these facts is that total calories are more relevant than dietary fat and people are merely replacing the energy from fat with carbohydrates. Moreover, we need to take into account the trends toward a more sedentary lifestyle with less energy expenditure. Alternatively, methodological problems, including the underreporting of energy and fat intake, may complicate understanding of the relationship between fat intake and obesity. However, gene-diet interactions such as those observed in the

TABLE 2  Indicators of obesity in Spanish adults by dietary fat intake and APOA5-1131T>C SNP

<table>
<thead>
<tr>
<th>Low fat²</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>TC+CC</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.4 ± 0.30</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>36.2 ± 0.37</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>98.9 ± 0.79</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>111.4 ± 0.60</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. n = 1296. *Pvalue after adjustment for age, sex, tobacco smoking, nutrition center, and regular alcohol consumption.

2 Fat intake less than or equal to (low fat) or greater than (high fat) than the mean of 98 g/d.
In the current population, we found a consistent gene-diet interaction between the -1131T\textsuperscript{C} SNP and total fat intake for obesity-related traits, including BMI and waist and hip circumferences. As was shown in the study by Corella et al. (15), participants homozygous for the -1131T major allele exhibited the expected positive association between fat intake and obesity. Conversely, in individuals carrying the APOA5 -1131C minor allele (~12% of this population), higher fat intakes were not associated with higher anthropometric measures. Based on the fact that most participants do not carry the minor allele of the SNP, these results could indicate that for the majority of the population, fat intake, particularly saturated fat, is obesogenic. However, the segment of the population carrying the C allele APOA5-1131T > C, appear to be more resistant to the obesogenic effects of fat intake.

Mechanisms to explain the observed differences in APOA5 -1131T allele-specific responses to fat for obesity outcomes can be hypothesized, but not proven. Impaired efficiency of ribosomal translation is linked to the -1131C allele and may lead to a reduced LPL-mediated TG uptake into adipocytes (40). Other potential mechanisms could be linked to a differential regulation of the APOA5 gene by thyroid hormones or PPAR (41,42). Support for mechanisms involving a relationship between PPAR and APOA5 for fat modulation of obesity-related traits is provided by evidence from other gene-diet interaction studies. For example, the PPAR\gamma proline to alanine substitution SNP (Pro12Ala) modifies the association between MUFA intake and obesity (43). Considering these results, a nutrigenomic approach may be useful in dietary intervention for the prevention and treatment of obesity, with fat intake recommendations tailored to the individual genotype.

Although our replication of the previously demonstrated gene-nutrient interaction for obesity (15) increases its scientific credibility, the current study also provides data for a gene-diet interaction for a second outcome, that of plasma TG. As previously indicated, APOA5 has emerged as one of the most solid candidate genes affecting plasma TG in the general population (7) and our results in a Mediterranean population are consistent with those in other populations (44,45). As in earlier studies, carriers for the C allele at the APOA5 -1131T > C had higher TG compared with TT participants. Mechanisms underlying the association between APOA5 and TG are incomplete but include APOA5 enhancement of lipolysis via LPL activation and APOA5-facilitated clearance of TG-rich lipoprotein particles (16,40,46–49).

Despite the general consensus regarding the association of APOA5 variant alleles with higher TG concentrations, the strength of these associations is variable across populations. Our study provides evidence that nutrients may interact with genotype to modulate associations with TG. In the present study may also provide a plausible explanation for the current obesity trends.

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population, characterized by a high intake of MUFA, a significant gene-diet interaction was found between total fat intake and plasma TG-rich lipoprotein concentrations. Fat intake was inversely related to plasma TG and VLDL concentrations in C carriers, suggesting that these individuals were more resistant to the effects of fat intake. Our results are strengthened by our detection of interaction between APOA5 -1131T > C and fat evaluated either categorically (Fig. 1) or continuously (Fig. 2). Further, the relationship for TG was not eliminated by adjustment for BMI and carbohydrate intake, suggesting that it was not mediated by obesity or a high intake of carbohydrate.

Although our results for interactions between APOA5 genetic variability and fat intake for obesity are consistent with earlier work, the data for interactions affecting TG are inconclusive. Our results are consistent with those of Mattei et al. (16) in which minor allele carriers consuming high fat were protected against higher TG. In contrast, Lai et al. (19) reported that a high intake of PUFA in minor allele carriers was associated with higher plasma TG in U.S. whites. Population differences may be related to heterogeneity in age and genetic background but may also be related to differences in dietary patterns and sources of dietary fat in U.S. and Spanish diets. For example, high MUFA intake in Spanish populations is supplied by olive oil, which is less commonly consumed in the US.

In summary, we have replicated previous gene-diet interactions between -1131T > C SNP in the APOA5 gene and total fat intake for obesity traits and also detected a significant interaction for plasma TG and VLDL. The minor allele appears to be protective for both the accumulation of fat and for higher plasma TG in people consuming higher levels of dietary fat. Evidence of APOA5 genotype modulation of the effect of dietary fat on 2 important CVD risk factors (TG and BMI) strengthens the hypothesis that gene-diet interactions underlie associations between APOA5 and cardiovascular risk. These results also highlight the potential usefulness of a nutrigenomic approach in optimizing a dietary intervention for the prevention of obesity and CVD based on fat intake recommendations that are tailored to the individual genotype.

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M.G. and J.M.O. designed research; M.G. and J.M.O. conducted research; C.E.S., Y.C.L., C.S.M., J.C.B., and M.G. provided essential materials; C.E.S. and M.G. analyzed data; M.G., C.E.S., and J.M.O. wrote the paper; and M.G. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


