Genetic Variation at the ApoA-IV Gene Locus and Response to Diet in Familial Hypercholesterolemia

Rafael Carmena-Ramon, Juan F. Ascaso, Jose T. Real, Jose M. Ordovas, Rafael Carmena

Abstract—Plasma lipid response to dietary fat and cholesterol is, in part, genetically controlled. The apolipoprotein A-IV (apoA-IV protein; APOA4, gene) has been shown to influence the response to dietary changes in normolipidemic individuals. The response to diet in subjects with familial hypercholesterolemia (FH) is also variable, and no studies are available on the influence of APOA4 mutations on dietary response in these subjects. We studied the effect of 2 common apoA-IV genetic variants (Gln360→His and Thr347→Ser) on the lipid response to the National Cholesterol Education Program type I (NCEP-I) diet in 67 FH heterozygotes (43 women and 24 men). Subjects were studied at baseline (after consuming for 1 month a diet with 35% fat [10% saturated] and 300 mg/d cholesterol) and after 3 months of consuming a low-fat diet. No sex-related differences were found, and results were combined for men and women. The APOA4-360 mutation was assessed in 67 subjects, 51 with genotype 1/1 and 16 with genotype 1/2. The APOA4-2 allele was associated with marginally significantly lower (P=0.049) low density lipoprotein (LDL) cholesterol levels and significantly lower (P=0.027) apoB levels independent of diet effects. After consuming an NCEP-I diet, carriers of the APOA4-2 allele showed a significantly lower reduction in apoB concentration (6.2%) than 1/1 subjects (14.1%; P=0.036); however, no significant differences in response were noted for LDL cholesterol. The APOA4-347 mutation was assessed in 63 individuals, 44 with the A/A allele and 19 with the A/T and T/T alleles. No significant differences were observed in baseline or post–NCEP-I diet values for these 2 groups in total, LDL, and high density lipoprotein cholesterol and plasma apoB levels. After dietary intervention, A/A individuals showed significant reductions in plasma triglyceride and very low density lipoprotein cholesterol levels; no changes were found in carriers of the T allele. Haplotype analysis suggested that in these FH subjects, the APOA4-360-2 allele was associated with lower plasma lipid levels during the NCEP-I diet period, whereas no significant effects were observed for the APOA4-347-T allele.

Key Words: apolipoprotein A-IV □ genetic variation □ familial hypercholesterolemia □ diet

Human apolipoprotein A-IV (apoA-IV) is synthesized as a 46-kDa glycoprotein in the small intestine and secreted with intestinal lipoproteins during fat absorption. Although the precise function of apoA-IV is still unknown, it has been proposed that apoA-IV plays an important role in the metabolism of triglyceride (TG)-rich lipoproteins and HDLs. Several studies have shown that apoA-IV mediates the activation of lipoprotein lipase (promoting clearance of TG-rich lipoproteins and formation of HDL) and activates lecithin:cholesterol acyltransferase, an essential step in reverse cholesterol transport. It has also been shown that apoA-IV can regulate cholesterol ester transfer mediated by the cholesterol ester transfer protein between HDL and LDL.

Several genetic variants of apoA-IV have been identified. Initially, a common variant was found to be caused by a Gln→His substitution at amino acid 360. The frequency of the His isoform (APOA4-2 allele) varies worldwide, from being completely absent in Japan and among American Indians to 0.11 in Iceland. Some population studies have shown that the APOA4-2 allele is associated with a less atherogenic profile, although other studies have not confirmed this observation.

In dietary intervention trials, carriers of this allele have been shown to be less responsive to dietary changes, in terms of LDL cholesterol (LDL-C), than carriers of the APOA4-1/1 (Gln/Gln) alleles. More recently, the APOA4-2 allele was associated with Alzheimer’s disease.

The application of DNA sequence analysis has revealed additional variation at this locus. Thus, a variant within the APOA4-1 allele due to an A→T substitution in the coding region results in a Thr347→Ser substitution. The frequency of this mutation ranges from being absent in the Japanese population to 0.12 in Asian Indians and 0.21 in whites. Data concerning the effect of the 347 mutation on the dietary response of plasma lipids and lipoproteins in healthy young men showed a greater decrease in total cholesterol, LDL-C, and apoB when subjects switched from a diet high in saturated fat to the National Cholesterol Educational Program type I (NCEP-I) diet.
Changes in plasma lipid levels in response to dietary modifications vary greatly among individuals in the general population and are, in part, genetically controlled. The response to diet in subjects with familial hypercholesterolemia (FH) is also highly variable; however, no studies have been performed on the influence of apoA-IV mutations on dietary response in these subjects. Our aim in this study was to investigate the effect of the genetic variants of apoA-IV at the 360 and 347 positions on the response to a hypolipidemic diet in a group of heterozygous subjects with FH.

Methods

Subjects

A total of 67 heterozygous subjects with FH were studied. There were 43 women (mean age, 37.6 ± 16.9 years) and 24 men (mean age, 34.6 ± 15.2 years). The subjects were members of FH pedigrees previously diagnosed at the Lipid Clinic, Hospital Clinico Universitario, Valencia, Spain. Diagnosis of FH was established by family history, lipid values (total cholesterol >6.2 mmol/L or LDL-C >5.2 mmol/L), and genetic characterization of the LDL-receptor (LDLR) locus according to a previously described protocol. We found 13 subjects with major rearrangements of the LDLR gene, 39 with small point mutations and 17 with an indirect genetic diagnosis. In summary, we found a total of 17 subjects with null mutations (receptor absent), 18 with receptor-defective mutations, and 34 in whom the specific LDLR phenotype associated with their genotype remains to be elucidated (undefined). The 2 most prevalent mutations were the 111 INS A and the C358 Y mutations (J.T.R. et al, unpublished observations, 1998).

Subjects were recruited by random selection during their regular visits to the clinic during 6 consecutive months in 1995; of 90 subjects selected, 76 agreed to participate and 67 completed the study. Because of the relatively young mean age of our sample, atherosclerotic cardiovascular disease was clinically present in 8 subjects: one 70-year-old woman and one 68-year-old man had documented myocardial infarction; one 61-year-old man had angina pectoris; one 62-year-old woman had angina pectoris and peripheral vascular disease; and 4 women (ages 51 to 62 years). The two men also had evidence of cardiovascular disease on an exercise ECG. There were 19 current smokers (9 women and 10 men). Hypertension was diagnosed if blood pressure levels were above 140/90 mm Hg on more than 2 occasions and was present in 4 women. Non-insulin-dependent diabetes mellitus was present in 2 women. Informed consent was obtained from all subjects. The study protocol was reviewed and approved by the Clinical Research Committee of the Hospital Clinico Universitario. Table 1 lists baseline characteristics of the subjects.

Study Protocol

The study was conducted on an outpatient basis under supervision of physicians at the clinic and a clinical dietitian. On inclusion, subjects entered a 4-week baseline period in which they interrupted their NCEP-I diet and switched to a diet containing 35% daily energy derived from fat (10% saturated fat) and 300 mg/d of cholesterol. All medications known to influence plasma lipid levels were discontinued during this period. At the end of the baseline period, venous blood was drawn for plasma lipid and lipoprotein measurements and results were considered as baseline values. Subjects then entered the low-fat diet period, which lasted for 3 months, during which they did not take medications known to influence lipid levels and followed the NCEP-I diet. To ensure compliance during both the run-in and diet periods, the 24-hour diet recall questionnaire and the food frequencies checklist were used as previously described. Subjects were instructed to maintain their regular level of physical exercise and lifestyle. All subjects visited the clinic monthly for weight and blood pressure measurements and monitoring of dietary compliance. Caloric adjustments were made according to changes in body weight. Body mass index (BMI) was calculated (as kg/m2) at the start and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n=24)</th>
<th>Women (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.6±15.2</td>
<td>37.6±16.9</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.52±4.52</td>
<td>24.20±3.87</td>
</tr>
<tr>
<td>Smokers*</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>HBP†</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>NIDDM, n</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Corneal arcus,</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Xanthomata, n</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Xanthelasma, n</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>CVD, ‡</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

CVD indicates cardiovascular disease; HBP, high blood pressure; and NIDDM, non–insulin-dependent diabetes mellitus.
*Current smokers of ≥1 cigarettes per day and ex-smokers of <1 year.
†HBP was defined as ≥140/90 mm Hg.
‡Presence of clinical signs and/or symptoms of cardiovascular disease.

remained constant throughout the experimental period. At the end of the study period, blood was obtained from all subjects for lipid and lipoproteins measurements, DNA amplification, and genotyping.

Measurement of Plasma Lipids and Lipoproteins

Blood samples from fasting subjects were collected in Vacutainer tubes containing EDTA by venipuncture. Plasma was separated by low-speed centrifugation and stored at 4°C; samples were assayed within 24 hours of collection. Plasma total cholesterol and TG concentrations were determined by enzymatic methods and HDL cholesterol (HDL-C) was measured by precipitation with polyethylene glycol, and the LDL-C concentration was calculated from the total cholesterol, TG, and HDL-C concentrations by using the Friedewald formula and by direct measurement of VLDL cholesterol (VLDL-C) after sequential ultracentrifugation at 40 000 rpm for 18 hours. None of our subjects had TG concentrations above 3.38 mmol/L. Total plasma apoB-100 was measured by radial immunodiffusion.

Analysis of ApoA-IV Genotype

DNA was extracted from 10 ml of EDTA-containing blood. Fifty nanograms was used for amplification by polymerase chain reaction. The presence of the APOA4-347 variant was studied in 67 subjects with FH using the Hinf I restriction enzyme, which allows identification of 3 genotypes: 1/1, 1/2, and 2/2. Fifty-one subjects (32 women and 19 men) were 1/1, 16 (11 women and 5 men) were 1/2, and none were homozygous 2/2.

The APOA4-347 polymorphism was assessed in 63 subjects with FH (41 women and 22 men) using Hinf I (Promega), which allows identification of 3 genotypes: A/A, A/T, and T/T. Forty-four subjects were carriers of the heterozygous form of the 347Thr allele (allele A), and 19 had the 347Ser allele (allele T) in its heterozygous (18 subjects, 11 women and 7 men) or homozygous (1 woman) form (this last subject was combined with carriers of the heterozygous form in the statistical analyses).

Statistical Analyses

SPSS for Windows version 7.5.1 was used for all statistical analyses. All continuous variables except TG were normally distributed, as assessed by the Kolmogorov-Smirnov test. TG values were logarithmically transformed to achieve approximately normal distribution, and statistical tests were done on the transformed values. In this population, no statistically significant differences were demonstrated between men and women, and all analyses were performed in the entire group. All data presented in the text and tables are mean ± SD. One-way ANOVA was used to test the difference between genotypes at baseline and after the NCEP-I diet period. The general linear
model for repeated measures was used to test the significance of the lipid response to dietary intervention as well as gene-diet interactions between apoA-IV genotypes and dietary response for each lipid variable examined. Age and BMI were used as covariates in these analyses.

**Results**

No differences were observed between men and women with respect to age, BMI, or baseline concentrations of plasma lipids. After intervention with the low-fat diet, there was a significant reduction in both sexes in total cholesterol, LDL-C, and total plasma apoB, with no significant changes in VLDL-C, TG, and HDL-C. Because no significant differences in response were observed between men and women, we present the data of plasma lipid changes for the total group (Table 2).

After subjects consumed the NCEP-I diet for 3 months, significant reductions ($P<0.0001$) in total cholesterol, LDL-C, and total plasma apoB concentrations were observed. The 15% reduction in total cholesterol and the 17% reduction in LDL-C levels are in agreement with previous responses to this type of diet reported for FH heterozygotes.26 Plasma TG, HDL-C, and VLDL-C concentrations did not change significantly (Figures 1 and 2).

**Effect of the Gln$^{360}$→His Variant on Plasma Lipids and Dietary Response**

Subjects were classified according to the presence (16) or absence (51) of the APOA4-2 allele, and each group was further subdivided according to LDLR phenotype (null, defective, or undefined). Mean values and the statistical significance of the difference between groups at each diet period and between diets for each group are shown in Table 3. Percent changes between diet periods are presented in Figure 1. To test the associations between the APOA4-360 polymorphism and plasma lipid levels, we analyzed data for both diet periods combined (global analysis) and for each diet period alone (baseline and NCEP-I). The global analysis revealed a marginally significant difference for LDL-C levels, with carriers of the APOA4-2 allele showing lower levels (1.45 and 1.36 g/L for the baseline and NCEP-I diet periods, respectively) than apoA-IV-1 subjects (1.70 and 1.46 g/L, $P=0.027$). In this case these effects were primarily due to significantly lower (0.23 g/L; $P=0.012$) apoB levels during the baseline period. No statistically significant genotype-related differences were demonstrated for total cholesterol, triglycerides, HDL-C, and VLDL-C. The only significant gene (APOA4-360)–diet interaction was observed for the apoB response. Carriers of the APOA4-2 allele had a diet-induced reduction in apoB levels (6.2%) that was significantly lower than that observed in subjects homozygous for the APOA4-1 allele (14.1%, $P=0.036$) (Figure 1). No significant differences were observed in the response to diet for apoB levels, with carriers of the APOA4-2 allele showing lower levels (1.45 and 1.36 g/L for the baseline and NCEP-I diet periods, respectively) than apoA-IV-1 subjects (1.70 and 1.46 g/L, $P=0.027$). In this case these effects were primarily due to significantly lower (0.23 g/L; $P=0.012$) apoB levels during the baseline period. No statistically significant genotype-related differences were demonstrated for total cholesterol, triglycerides, HDL-C, and VLDL-C. The only significant gene (APOA4-360)–diet interaction was observed for the apoB response. Carriers of the APOA4-2 allele had a diet-induced reduction in apoB levels (6.2%) that was significantly lower than that observed in subjects homozygous for the APOA4-1 allele (14.1%, $P=0.036$) (Figure 1). No significant differences were observed in the response to diet for

### Table 2. Plasma Lipid and ApoB Changes Observed in 67 Subjects With FH at Baseline and After 3 Months on NCEP-I Diet

<table>
<thead>
<tr>
<th>Lipid Variable</th>
<th>Baseline</th>
<th>After diet intervention</th>
<th>Change, %</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>8.90 ± 1.62</td>
<td>7.57 ± 1.51</td>
<td>-14.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>6.99 ± 1.53</td>
<td>5.77 ± 1.50</td>
<td>-17.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.15 ± 0.55</td>
<td>1.08 ± 0.58</td>
<td>-0.6</td>
<td>0.283</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.40 ± 0.41</td>
<td>1.32 ± 0.38</td>
<td>-0.6</td>
<td>0.060</td>
</tr>
<tr>
<td>VLDL-C, mmol/L</td>
<td>0.54 ± 0.33</td>
<td>0.45 ± 0.26</td>
<td>-16.7</td>
<td>0.068</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.64 ± 0.33</td>
<td>1.43 ± 0.25</td>
<td>-12.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Subjects included 43 women and 24 men.

*Minus signs indicate reduction.

†Baseline value vs value after NCEP-I diet.

![Figure 1](https://via.placeholder.com/150)

Figure 1. Percent changes for LDL-C, apoB, HDL-C, and TG induced by the NCEP-I diet. Data are presented for all subjects and for subgroups defined by the APOA4-360 polymorphism (1/1 and 1/2) and LDLR phenotype (null or defective). ■ indicates APOA4-360-1/1, LDLR null; □, APOA4-360-1/1, LDLR defective; ●, APOA4-360-1/2, LDLR null; and ○, APOA4-360-1/2, LDLR defective.
plasma total cholesterol, LDL-C, HDL-C, VLDL-C, and TG concentrations.

The percent changes between diet periods for LDL-C, apoB, HDL-C, and TG in the APOA4-360 and LDLR subgroups are shown in Figure 1. None of the between-group differences for LDL-C, HDL-C, and TG percent changes were significantly different from the mean percent decrease; however, as described above for the absolute changes, the percent reduction in apoB levels was significantly less in subjects with the APOA4-1/2 genotype, independently of the LDLR phenotype.

Effect of the Thr347→Ser Variant on Plasma Lipids and Dietary Response

Subjects were separated according to the presence (19) or absence (44) of the APOA4-T allele at the A4-347 codon. Each group was further subdivided according to LDLR phenotype (null or defective). Table 4 shows mean±SD plasma lipid and apoB levels at baseline and after the hypolipidemic diet period for each of these groups. No significant overall (both diet periods combined) differences were observed between groups for total cholesterol, LDL-C, HDL-C, and total plasma apoB levels. However, the TG and VLDL-C plasma levels after the NCEP-I diet period were significantly higher in the A/T+T/T group (1.37±0.92 and 0.56±0.44 mmol/L, respectively) than in the A/A group (0.96±0.35 and 0.41±0.15 mmol/L). No significant gene (APOA4-347)–diet interactions were observed between the 2 groups for any variable examined.

The percent changes between diet periods for LDL-C, apoB, HDL-C, and TG, by APOA4-347 and LDLR subgroups, are shown in Figure 2. None of the between-group differences for LDL-C, apoB, HDL-C, and TG percent changes were significantly different from the mean percent decrease.

Effect of the LDLR Phenotype on Plasma Lipids, Dietary Response, and Interaction With the APOA4 Locus

Within each apoA4 genotype group examined, subjects were classified according to 3 LDLR phenotypes. Subjects with LDLR mutations known to result in receptor-absent phenotypes were placed in the null group (n=17). Subjects in whom the LDLR mutation was shown to be associated with a defective LDLR were included in the defective group (n=18), and subjects for whom the specific LDLR phenotype associated with their genetic mutation has yet to be characterized were pooled together and classified as undefined (n=34). The lipid data are presented in Tables 3 and 4. Overall, the FH phenotype did not have a significant influence on total cholesterol, LDL-C, HDL-C, and TG levels (Table 3). However, VLDL-C levels were lower (P=0.032) in subjects in the defective group, primarily because of their significantly reduced levels observed after the NCEP-I diet period (P=0.019). During the NCEP-I diet period, TG levels were also significantly lower (P=0.024) in the FH-defective group. In terms of dietary response, analysis of 2-way interactions between LDLR subgroups and diet revealed no significant effects.

More complex gene (LDLR)–gene (APOA4)–diet interactions were also analyzed, and the results are presented in Tables 3 and 4. Significant 3-way interactions (LDLR–A4-360–diet) were demonstrated for total cholesterol (P=0.044) and LDL-C (P=0.039) (Table 3), whereas the only significant FH–A4-347–diet interaction was demonstrated for LDL-C (P=0.002).

Effect of APOA4 Haplotypes on Plasma Lipids and Dietary Response

Data on APOA4 haplotypes and their associations with plasma lipids and diet response are presented in Table 5. Three haplotypes were present in this population (A1, A2, and T1). The most common combined genotype was represented by the A1A1 haplotype (34 subjects), followed by the A1T1 (12), A1A2 (11), T1A2 (5), and T1T1 haplotypes (1). When the global effect of all haplotypes was analyzed versus plasma lipid variables, the only significant association was seen with total plasma cholesterol during the NCEP-I diet period (P=0.029), primarily because of a cholesterol-lowering effect associated with the A2 haplotype. Pairwise analyses between the different combined genotypes provided more significant information. During the baseline diet period, subjects with the A1A2 genotype displayed significantly lower apoB concentrations than those with the A1A1 or A1T1 genotype. Similar findings were observed for apoB concentrations measured during the NCEP-I diet period, but, in addition, subjects with the T1A2 genotype displayed higher apoB levels than A1A2 subjects. Significant genotype-
TABLE 3. Plasma Lipids and ApoB Changes at End of Each Dietary Period According to APOA4-360 Mutation in 67 FH Subjects

<table>
<thead>
<tr>
<th>Genotype A4</th>
<th>LDLR Phenotype</th>
<th>Diet</th>
<th>Lipid, mmol·L⁻¹</th>
<th>Lipid, mmol·L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TC</td>
<td>LDL-C</td>
</tr>
<tr>
<td>1/1 (n=51)</td>
<td>Null (n=11)</td>
<td>Baseline</td>
<td>9.08±1.57</td>
<td>7.47±1.28</td>
</tr>
<tr>
<td></td>
<td>Defective (n=13)</td>
<td></td>
<td>9.30±1.48</td>
<td>7.33±1.37</td>
</tr>
<tr>
<td></td>
<td>Undefined (n=27)</td>
<td></td>
<td>8.75±1.69</td>
<td>6.88±1.70</td>
</tr>
<tr>
<td></td>
<td>All (n=51)</td>
<td></td>
<td>9.00±1.61</td>
<td>7.16±1.54</td>
</tr>
<tr>
<td></td>
<td>Null (n=11)</td>
<td>NCEP</td>
<td>7.69±1.32</td>
<td>5.81±1.40</td>
</tr>
<tr>
<td></td>
<td>Defective (n=13)</td>
<td></td>
<td>8.33±1.78</td>
<td>6.45±1.76</td>
</tr>
<tr>
<td></td>
<td>Undefined (n=27)</td>
<td></td>
<td>7.49±1.31</td>
<td>5.83±1.39</td>
</tr>
<tr>
<td>1/2 (n=16)</td>
<td>Null (n=5)</td>
<td>Baseline</td>
<td>7.59±0.65</td>
<td>5.84±0.92</td>
</tr>
<tr>
<td></td>
<td>Defective (n=5)</td>
<td></td>
<td>10.28±1.83</td>
<td>7.66±1.19</td>
</tr>
<tr>
<td></td>
<td>Undefined (n=6)</td>
<td></td>
<td>8.13±1.43</td>
<td>6.07±1.65</td>
</tr>
<tr>
<td></td>
<td>All (n=16)</td>
<td></td>
<td>8.68±1.76</td>
<td>6.53±1.48</td>
</tr>
<tr>
<td></td>
<td>Null (n=5)</td>
<td>NCEP</td>
<td>6.27±0.70</td>
<td>4.58±0.64</td>
</tr>
<tr>
<td></td>
<td>Defective (n=5)</td>
<td></td>
<td>8.26±1.77</td>
<td>5.76±1.62</td>
</tr>
<tr>
<td></td>
<td>Undefined (n=6)</td>
<td></td>
<td>6.84±1.65</td>
<td>5.04±1.59</td>
</tr>
<tr>
<td></td>
<td>All (n=16)</td>
<td></td>
<td>7.06±1.56</td>
<td>5.14±1.38</td>
</tr>
</tbody>
</table>

Statistics*  
A4, both diets combined  
FH, both diets combined  
A4, baseline  
A4, NCEP  
FH, baseline  
FH, NCEP  
Diet  
Diet×A4  
Diet×FH  
Diet×A4×FH

Data are mean±SD.  
*The SPSS general linear model for repeated measures was used for these analyses using genotypes as between-subjects factors and lipid measurements as dependent variables. Age and BMI were used as covariates in all analyses.

related differences were also seen for total cholesterol and LDL-C (A1T1 versus A1A2), TG, and VLDL-C (A1T1 versus A1T2 and A1T1 versus A1A2). These data suggest that in this population, the A2 haplotype was associated with lower plasma levels for all lipid variables examined (total cholesterol, LDL-C, TG, VLDL-C, and apoB) compared with the A1 haplotype, whereas the T1 haplotype had a mild lipid-raising effect. No significant haplotype-diet interactions were demonstrated in this population.

Discussion

Plasma lipid response to dietary fat and cholesterol is, to a large extent, genetically controlled.23–25 The range of the response to changes in the amount or type of dietary fat and cholesterol varies among individuals.35–37 As a group, our FH subjects (Table 2) responded to the NCEP-I diet with 15% and 17% reductions in total cholesterol and LDL-C, respectively, in agreement with what has been previously reported.26

ApoA-I, apoB, and apoE are among the genetic loci that have been implicated in the variable lipidic response to dietary changes. Previous studies with apoA-IV conducted in healthy, young normolipidemic individuals17 and in a multicenter study including volunteers from the general population16 have shown that individuals carrying the APOA4-360-2 allele have an attenuated hypercholesterolemic response to the ingestion of a very-high-cholesterol diet and decreased LDL-C lowering in response to reductions in dietary saturated fat and cholesterol, respectively. Conversely, in a population study in Costa Rica,39 it was observed that urban carriers of the APOA4-2 allele had significantly lower HDL-C and apoA-I concentrations and a higher LDL-C/HDL-C ratio than rural APOA4-2 carriers or urban APOA4-1/1 carriers. Compared with a rural lifestyle, the urban lifestyle was characterized by increased smoking and intake of saturated fat. These data suggest a complex interaction between the APOA4 gene locus and environmental factors.

Few data are available on the effect of the 347Ser (T allele) mutation, a common variant of apoA-IV, on lipid response to diet. In a recent study32 in healthy young men, individuals with the 347Ser (T allele) mutation had a greater decrease in total cholesterol, LDL-C, and apoB levels when they
switched from a diet high in saturated fat to the NCEP-I diet, indicating that this mutation of APOA4 also influences the lipid response to dietary fat in normolipidemic individuals.

We investigated the effects of APOA4-360 and APOA4-347 variants on the lipid response to diet in subjects with FH, a population in which no data are yet available. Our study included 67 FH heterozygotes in whom the genetic defect at the LDLR gene locus had been previously assessed. During a 4-week run-in period, subjects consumed an average Spanish diet containing 35% of daily energy derived from fat (10% saturated, 15% monounsaturated, and 10% polyunsaturated fat) and 300 mg/d of cholesterol. Baseline lipid values were obtained at the end of this period.

We did not find differences in baseline lipid levels between carriers of the APOA4-2 allele and carriers of APOA4-1/1, a finding also reported by McCombs et al. On the other hand, baseline plasma apoB concentrations were significantly lower in our APOA4-2 subjects. For the APOA4-347 variant, no differences were found at baseline for any lipid or apolipoprotein level between carriers of the APOA4-347-T allele and individuals homozygous for APOA4-347-A. In cross-sectional studies, carriers of the APOA4-347-T allele have been reported to have lower levels of apoB and LDL-C. We did not observe this, and our findings agree with those of Jansen et al, who studied a group of healthy, young normolipidemic men. Hence, with the exception of lower total plasma apoB levels in our FH APOA4-2 subjects, the 2 apoA-IV mutations, when individually assessed, do not seem to influence baseline plasma lipid levels, and, in this regard, our FH subjects were similar to the normolipidemic individuals of similar genetic origin previously reported.

At the end of the NCEP-I diet period, the decrease in apoB levels was significantly lower in individuals carrying the APOA4-2 allele (6.2%) than in APOA4-1/1 individuals (14.1%). This hypolipidemic response was similar to what has been described for LDL-C when non-FH individuals consume a low-fat diet. Surprisingly, these effects observed for apoB were not paralleled by those observed for LDL-C, for which no allelic differences in dietary response could be demonstrated. These data suggest that dietary intervention may induce remodeling of the LDL particles in

---

**TABLE 4. Plasma Lipid and ApoB Changes at End of Each Dietary Period According to APOA4-347 Mutation in 63 FH Subjects**

<table>
<thead>
<tr>
<th>Genotype A4</th>
<th>LDLR Phenotype</th>
<th>Diet</th>
<th>TC, mmol · L⁻¹ · L⁻¹</th>
<th>LDL-C, mmol · L⁻¹ · L⁻¹</th>
<th>TG, mmol · L⁻¹ · L⁻¹</th>
<th>HDL-C, mmol · L⁻¹ · L⁻¹</th>
<th>VLDL-C, mmol · L⁻¹ · L⁻¹</th>
<th>ApoB, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A (n=44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null (n=12)</td>
<td>Baseline</td>
<td></td>
<td>8.22±1.34</td>
<td>6.63±1.36</td>
<td>1.01±0.58</td>
<td>1.29±0.47</td>
<td>0.46±0.26</td>
<td>1.61±0.38</td>
</tr>
<tr>
<td>Defective (n=8)</td>
<td></td>
<td></td>
<td>10.02±0.77</td>
<td>7.87±0.68</td>
<td>1.16±0.31</td>
<td>1.49±0.29</td>
<td>0.46±0.20</td>
<td>1.63±0.45</td>
</tr>
<tr>
<td>Undefined (n=24)</td>
<td></td>
<td></td>
<td>8.95±1.78</td>
<td>6.98±1.88</td>
<td>1.15±0.46</td>
<td>1.47±0.44</td>
<td>0.60±0.41</td>
<td>1.68±0.21</td>
</tr>
<tr>
<td>All (n=44)</td>
<td></td>
<td></td>
<td>8.93±1.61</td>
<td>7.05±1.62</td>
<td>1.11±0.47</td>
<td>1.42±0.42</td>
<td>0.53±0.34</td>
<td>1.65±0.36</td>
</tr>
<tr>
<td>Null (n=12)</td>
<td>NCEP</td>
<td></td>
<td>6.87±1.31</td>
<td>5.31±1.32</td>
<td>0.99±0.41</td>
<td>1.17±0.38</td>
<td>0.44±0.19</td>
<td>1.38±0.27</td>
</tr>
<tr>
<td>Defective (n=8)</td>
<td></td>
<td></td>
<td>8.08±1.59</td>
<td>5.94±1.51</td>
<td>1.09±0.32</td>
<td>1.56±0.32</td>
<td>0.44±0.16</td>
<td>1.37±0.19</td>
</tr>
<tr>
<td>Undefined (n=24)</td>
<td></td>
<td></td>
<td>7.61±1.39</td>
<td>5.89±1.50</td>
<td>0.91±0.34</td>
<td>1.31±0.36</td>
<td>0.37±0.12</td>
<td>1.43±0.27</td>
</tr>
<tr>
<td>All (n=44)</td>
<td></td>
<td></td>
<td>7.48±1.44</td>
<td>5.73±1.44</td>
<td>0.96±0.35</td>
<td>1.31±0.37</td>
<td>0.41±0.15</td>
<td>1.40±0.25</td>
</tr>
<tr>
<td>A/T or T/T (n=19)</td>
<td></td>
<td></td>
<td>9.49±1.61</td>
<td>7.62±1.35</td>
<td>1.32±0.52</td>
<td>1.31±0.45</td>
<td>0.64±0.19</td>
<td>1.50±0.14</td>
</tr>
<tr>
<td>Null (n=5)</td>
<td>Baseline</td>
<td></td>
<td>9.33±2.05</td>
<td>7.14±1.61</td>
<td>1.25±0.87</td>
<td>1.50±0.30</td>
<td>0.54±0.40</td>
<td>1.67±0.31</td>
</tr>
<tr>
<td>Defective (n=10)</td>
<td></td>
<td></td>
<td>8.37±1.45</td>
<td>6.36±1.48</td>
<td>1.51±0.71</td>
<td>1.45±0.44</td>
<td>0.69±0.33</td>
<td>1.82±0.30</td>
</tr>
<tr>
<td>Undefined (n=4)</td>
<td></td>
<td></td>
<td>9.15±1.78</td>
<td>7.10±1.57</td>
<td>1.32±0.73</td>
<td>1.43±0.38</td>
<td>0.60±0.33</td>
<td>1.65±0.28</td>
</tr>
<tr>
<td>All (n=19)</td>
<td></td>
<td></td>
<td>7.99±1.25</td>
<td>5.54±1.63</td>
<td>2.16±1.40</td>
<td>1.41±0.60</td>
<td>0.98±0.65</td>
<td>1.44±0.16</td>
</tr>
<tr>
<td>Null (n=5)</td>
<td>NCEP</td>
<td></td>
<td>8.49±1.92</td>
<td>6.60±1.89</td>
<td>1.04±0.53</td>
<td>1.49±0.36</td>
<td>0.37±0.24</td>
<td>1.55±0.33</td>
</tr>
<tr>
<td>Defective (n=10)</td>
<td></td>
<td></td>
<td>7.05±2.10</td>
<td>5.29±1.93</td>
<td>1.20±0.46</td>
<td>1.24±0.36</td>
<td>0.54±0.21</td>
<td>1.42±0.30</td>
</tr>
<tr>
<td>Undefined (n=4)</td>
<td></td>
<td></td>
<td>8.04±1.80</td>
<td>6.02±1.81</td>
<td>1.37±0.92</td>
<td>1.41±0.38</td>
<td>0.56±0.44</td>
<td>1.49±0.28</td>
</tr>
</tbody>
</table>

Statistics:
- A4, both diets combined
  - 0.344 ± 0.663
  - 0.058 ± 0.595
  - 0.084 ± 0.548
- FH, both diets combined
  - 0.962 ± 0.862
  - 0.313 ± 0.269
  - 0.041 ± 0.723
- A4, baseline
  - 0.624 ± 0.901
  - 0.263 ± 0.930
  - 0.494 ± 0.969
- A4, NCEP
  - 0.192 ± 0.493
  - 0.034 ± 0.379
  - 0.037 ± 0.218
- FH, baseline
  - 0.868 ± 0.672
  - 0.893 ± 0.261
  - 0.500 ± 0.403
- FH, NCEP
  - 0.988 ± 0.954
  - 0.019 ± 0.144
  - 0.013 ± 0.656
- Diet
  - <0.0001
  - <0.0001
  - 0.397 ± 0.208
  - 0.157 ± <0.0001
- Diet×A4
  - 0.289 ± 0.463
  - 0.392 ± 0.404
  - 0.400 ± 0.178
- Diet×FH
  - 0.939 ± 0.461
  - 0.060 ± 0.476
  - 0.171 ± 0.363
- Diet×A4×FH
  - 0.101 ± 0.002
  - 0.361 ± 0.277
  - 0.106 ± 0.799

Data are mean ± SD.

*The SPSS general linear model for repeated measures was used for these analyses using genotypes as between-subjects factors and lipid measurements as dependent variables. Age and BMI were used as covariates in all analyses.
TABLE 5. Plasma Lipid and ApoB Changes at End of Each Dietary Period According to APOA4 Haplotype in 63 FH Subjects

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>n</th>
<th>Diet</th>
<th>TC</th>
<th>LDL-C</th>
<th>TG</th>
<th>HDL-C</th>
<th>VLDL-C</th>
<th>ApoB, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1A1</td>
<td>34</td>
<td>Baseline</td>
<td>8.94±1.61</td>
<td>7.16±1.57</td>
<td>1.11±0.45</td>
<td>1.34±0.41</td>
<td>0.54±0.35</td>
<td>1.69±0.38†</td>
</tr>
<tr>
<td>A1T1</td>
<td>12</td>
<td></td>
<td>9.15±1.67</td>
<td>7.11±1.57</td>
<td>1.23±0.52</td>
<td>1.47±0.34</td>
<td>0.56±0.24</td>
<td>1.69±0.24‡</td>
</tr>
<tr>
<td>A1A2</td>
<td>11</td>
<td></td>
<td>8.32±1.44</td>
<td>6.18±1.26</td>
<td>0.97±0.50</td>
<td>1.59±0.39</td>
<td>0.43±0.24</td>
<td>1.40±0.19§</td>
</tr>
<tr>
<td>T1A2</td>
<td>5</td>
<td></td>
<td>9.43±2.31</td>
<td>7.27±1.81</td>
<td>1.58±1.21</td>
<td>1.43±0.52</td>
<td>0.72±0.55</td>
<td>1.55±0.40</td>
</tr>
<tr>
<td>T1T1</td>
<td>1</td>
<td></td>
<td>7.70</td>
<td>6.05</td>
<td>1.23</td>
<td>0.98</td>
<td>0.57</td>
<td>1.78</td>
</tr>
<tr>
<td>A1A1</td>
<td>34</td>
<td>NCEP</td>
<td>7.62±1.37</td>
<td>5.92±1.41</td>
<td>0.99±0.35‡</td>
<td>1.29±0.37‡</td>
<td>0.42±0.15</td>
<td>1.46±0.26‡</td>
</tr>
<tr>
<td>A1T1</td>
<td>12</td>
<td></td>
<td>8.22±1.74†</td>
<td>6.28±1.77†</td>
<td>1.46±1.08†</td>
<td>1.42±0.40‡</td>
<td>0.62±0.50</td>
<td>1.49±0.28‡</td>
</tr>
<tr>
<td>A1A2</td>
<td>11</td>
<td></td>
<td>6.62±1.16†</td>
<td>4.86±0.95†</td>
<td>0.88±0.27†</td>
<td>1.29±0.40†</td>
<td>0.37±0.15</td>
<td>1.27±0.12†§</td>
</tr>
<tr>
<td>T1A2</td>
<td>5</td>
<td></td>
<td>8.01±2.04</td>
<td>5.74±2.06</td>
<td>1.16±0.59</td>
<td>1.50±0.47</td>
<td>0.42±0.27</td>
<td>1.56±0.33§</td>
</tr>
<tr>
<td>T1T1</td>
<td>1</td>
<td></td>
<td>5.66</td>
<td>4.26</td>
<td>1.19</td>
<td>0.85</td>
<td>0.54</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Statistics* Haplotype, diets combined

- Haplotype, baseline: 0.146 ± 0.164, 0.058 ± 0.362, 0.331 ± 0.098
- Haplotype, NCEP: 0.554 ± 0.342, 0.692 ± 0.253, 0.628 ± 0.072
- Diet: <0.0001, <0.0001, 0.208 ± 0.157, 0.057 ± 0.001
- Diet × haplotype: 0.462 ± 0.540, 0.392 ± 0.272, 0.442 ± 0.067

Data are mean±SD. Differences for values within each diet period sharing the same symbol (†, ‡, §) were statistically significant (P<0.05).

*The SPSS general linear model for repeated measures was used for these analyses using haplotypes as between-subjects factors and lipid measurement as dependent variables. Age and BMI were used as covariates in all analyses.

Subjects with FH; however, this hypothesis needs to be tested in a larger population.

APOA4-2 has a slower catabolic rate, has more α-helical structure, is more stable in solution, is more hydrophobic, and binds to lipoproteins with higher affinity than APOA4-1.40 These properties of APOA4-2 could limit the adsorption of apoC-II and inhibit lipoprotein lipase activity. In this way, the formation and hepatic clearance of chylomicron remnants would be delayed, decreasing the amount of cholesterol reaching the liver in the postprandial state and causing less downregulation of hepatic LDLRs. This mechanism could explain the findings observed in APOA4-2 subjects, in whom an absence or attenuation of the lipid response to diets with a high or low fat content, respectively, has been reported.16,17

Concerning the APOA4-347 mutation, no significant allelic differences were related to the plasma lipid response to the low-fat diet in these subjects. These findings conflict with those of Jansen et al22 in healthy young men, because our FH subjects carrying the APOA4-347-T allele mutation did not have a greater decrease in total cholesterol, LDL-C, and apoB than homozygous carriers of the APOA4-347-A allele after consuming a low-fat diet, nor were their plasma TG levels lowered. Hence, the coexistence of an LDLR mutation blunts the lipid response to a low-fat diet observed in normolipemic APOA4-347-T individuals. The precise mechanism by which the APOA4-347-T mutation regulates a different response to dietary fat in normolipemic individuals is unknown. The substitution of serine for threonine at position 347 of apoA-IV produces changes in the secondary structure of the protein and a slight increase in hydrophilic profile in this position, which could result in a decrease in its affinity for lipoproteins.40 As a consequence, the exchange with apoC-II would be facilitated, increasing the activation of lipoprotein lipase and accelerating the clearance of chylomicron remnants. In the postprandial state, this would increase the amount of cholesterol reaching the liver and would increase downregulation of the LDLRs. The consumption of a fat-rich diet would increase the LDL-C in carriers of the mutation, whereas consumption of a low-fat diet should do the opposite. It seems plausible that in FH subjects carrying the 347Ser mutation, like the ones we studied, the low-fat diet fails to upregulate the LDLRs and no differences with 347Thr individuals are observed.

Three haplotypes (A1, A2, and T1) were observed in this population, resulting in 5 different combined genotypes. During the baseline high-fat diet period, the only significant difference between these genotypes was observed for apoB levels. The presence of the A1A2 genotype was associated with lower apoB levels compared with A1A1 or A1T1. These data suggest that the presence of the A2 haplotype was associated with lower apoB concentrations, with little or no effect resulting from the T1 haplotype. During the NCEP-I diet, a number of significant effects were noted. Overall, the presence of the A2 haplotype was associated with lower lipid concentrations, whereas the presence of T1 appeared to have a moderate increasing effect on plasma lipid concentrations, as suggested by the pairwise comparisons. The small representation of the less common genotypes precluded the finding of more significant associations in the global analysis. Our data agree with the findings reported by Saha et al31 regarding the A2 haplotype; however, these authors also found a significant LDL-C-lowering effect associated with the T1 haplotype. These differences may be due to the presence of the FH phenotype in our subjects. It is also possible that the differences in protocols, fasting samples in our study, and postprandial samples in the study of Saha et al could be in part responsible for these differences. Moreover, it is also possible that the 347 variant is not a causative mutation but is
in linkage disequilibrium with another mutation in the APOA4 or any other neighboring gene. If this is the case, this marker may have different effects in subjects with a different genetic background. Overall, the haplotype data showed more clearly the hypolipemic effect associated with the APOA4-360-2 allele; however, the haplotype analysis did not provide any additional information about gene–diet interactions compared with the single-allele analysis.

The inclusion of the LDLR phenotype in the analysis provided little additional information, probably because of limitations imposed by the small sample size (although the sample was large for this type of subject) and the relatively high number of subjects with an unknown LDLR phenotype. The major findings related to complex 3-way interactions, suggesting gene–gene–diet interactions; however, larger, well-controlled studies on FH subjects should be conducted to confirm these findings.

In conclusion, we showed that in individuals with FH, the consumption of an NCEP-I type diet results in significant decreases in LDL-C and apoB, with no significant effects on HDL-C and TG concentrations. Moreover, we observed a dramatic variability in response, similar to that observed in previous studies, and some of this variability appears to be associated with the APOA4 gene locus. More specifically, in subjects homozygous for the APOA4-1 variant, consumption of a low-fat diet resulted in greater apoB lowering than observed in APOA4-2 subjects. This type of response resembles what has been reported in the general normolipidemic population for LDL-C concentrations. On the other hand, in these subjects the APOA4-347-T allele was not associated with variability in LDL-C response. This is in contrast to the greater decrease in LDL-C observed when healthy nonfamilial individuals carrying such a mutation consumed a low-fat diet.22 Our results suggest the existence of a gene–diet–gene interaction between the LDLR and APOA4 genes, and these 2 loci account for a minor proportion of the observed variability and may help explain some of the differences in the response to diet observed among individuals with FH.

Acknowledgments
This work was supported by grant HL 54776 (J.M.O.) from the National Institutes of Health, Bethesda, Md. We are indebted to Maria Antonia Priego and Gracia Romero for their help with the study.

References
Genetic Variation at the ApoA-IV Gene Locus and Response to Diet in Familial Hypercholesterolemia
Rafael Carmena-Ramon, Juan F. Ascaso, Jose T. Real, Jose M. Ordovas and Rafael Carmena

doi: 10.1161/01.ATV.18.8.1266
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/8/1266

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/