Effect of 360His mutation in apolipoprotein A-IV on plasma HDL-cholesterol response to dietary fat

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Abstract In order to determine whether genetic variability of apolipoprotein (apo) A-IV is responsible for the improvement in lipid profile when dietary saturated fats are replaced by carbohydrates or monounsaturated fats, 41 healthy male subjects were studied: 33 were homozygous for the 360Gln allele and 8 were heterozygote carriers of the 360His allele. These were administered three consecutive 4-week diets. The first was a diet rich in saturated fat (SAT diet, with 38% fat, 20% saturated. This was followed by a low fat diet (NCEP-I, with < 30% fat, < 10% saturated). The final diet was rich in monounsaturated fat (MUFA diet, with 38% fat, 22% monounsaturated). There was no difference in plasma lipid and apolipoprotein levels of both groups of individuals after consuming the SAT diet. Switching from this diet to the NCEP-I diet, carriers of the 360His allele presented a greater decrease in high density lipoprotein-cholesterol (HDL-C) (−10 vs. −1 mg/dL, $P < 0.004$) and apoA-I levels (−19 vs. −8 mg/dL, $P < 0.037$). Similarly, replacement of carbohydrates by monounsaturated fats produced a greater increase in HDL-C (9 vs. 1 mg/dL, $P < 0.003$) and apoA-I levels (9 vs. 2 mg/dL, $P < 0.036$) in carriers of the 360His mutation. Lecithin: cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities and apoA-IV levels were also measured. However, no genotype-related differences were observed for these parameters. Our results suggest that variability in HDL-C and apoA-I response to diet is, at least partially, determined by the 360His mutation of apoA-IV.—**Jansen, S., J. Lopez-Miranda, J. M. Ordovas, J. L. Zambrana, C. Marin, M. A. Ostos, P. Castro, R. McPherson, F. Lopez Segura, A. Blanco, J. A. Jimenez Pereperez, and F. Perez-Jimenez.** Effect of 360His mutation in apolipoprotein A-IV on plasma HDL-cholesterol response to dietary fat. *J. Lipid Res.* 1997. **38:** 1995–2002.

Supplementary key words apoA-IV polymorphism • dietary fatty acids • lipid response to dietary fat

Apolipoprotein (apo) A-IV is synthesized in the intestine (1) and has been associated with fat absorption (1–4) Moreover, it has been shown to activate the enzyme lecithin: cholesterol acyltransferase (LCAT) in vitro (5) and regulate activity of cholesteryl ester transfer protein (CETP) (6) and lipoprotein lipase (LPL) (7). In humans, isoelectric focusing and immunoblotting have identified up to eight different isoforms of this apolipoprotein (apoA-IV-0 to A-IV-7) (8–10). The most prevalent is apoA-IV-1, with a frequency of 0.885–0.986 (11, 12). Another prevalent isoform is apoA-IV-2, due to a G to T substitution in the gene that causes the appearance of histidine (CAT) instead of glutamine (CAG) in amino acid 360 of the protein (13). Its frequency in the different studies varies from 0.05 to 0.117 (8, 11). The 360His allele is present mainly in Caucasian populations, and it is significantly less frequent or undetectable in other populations (8). There is also a rare variant in apoA-IV-2 caused by a deletion of 12 base pairs that results in the loss of amino acids 362–365 (one glutamic acid and three glutamines) from the mature protein (14).

Several population studies have examined the association of apoA-IV (Gln/His) variant at codon 360 with plasma lipid levels. The conclusions of these studies are highly variable. Whereas some reported no significant differences between genotypes (10, 15–21), others observed elevated HDL-C (22, 23) or apoA-I levels (24) in subjects carrying the His allele. These differences could possibly be due to interactions between this isoform and dietary factors within each of the population studied. As apoA-IV is primarily found in HDL (25, 26), the main objective of this study was to determine to what extent

Abbreviations: apo, apolipoprotein; LCAT, lecithin: cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; HDL-C, high density lipoprotein cholesterol; BMI, body mass index; SFA, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NCEP, National Cholesterol Education Program; LDL-C, low density lipoprotein cholesterol.

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the apoA-IV 360His variant is associated with different HDL-C responses to changes in dietary fat.

SUBJECTS AND METHODS

Subjects and diets

The initial study population was comprised of 115 Caucasian male students from the University of Cordoba. All had a comprehensive medical history, physical examination, and clinical chemistry analysis before enrollment. Subjects who were selected were under 30 years of age with total cholesterol plasma levels lower than 5.7 mmol/L (220 mg/dL) on their usual diets, with no evidence of any chronic illness (such as hepatic, renal, thyroid, or cardiac dysfunction), and no unusually high levels of physical activity. The final group consisted of 41 of these subjects who volunteered to participate in the study. Of these, 33 were homozygote for the 360Gln allele and 8 were heterozygote carriers of the 360His allele. Thirty-three subjects presented the apoE 3/3 genotype (29 360Gln/Gln and 4 360Gln/His), 6 the 3/2 genotype (4 360 Gln/Gln and 2 360Gln/His), one the 3/4 genotype (360Gln/His), and another the 2/4 genotype (360Gln/His). No individual had a family history of coronary heart disease and none had received medication or vitamin supplements in the 6 months prior to the start of the study. Dietary information, including alcohol consumption, was collected over 7 consecutive days. None of the subjects was a usual drinker, and 19 were smokers (15 360Gln/Gln and 4 360Gln/His). Individual energy requirements were calculated by taking into consideration each subject's physical activity. Mean body mass index (BMI) was calculated as 24.5 ± 2.6 kg/m^2 (mean ± SD) at the start and remained constant throughout the experimental period. Subjects were encouraged to maintain regular physical activity and life style, and were asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits, and alcohol consumption or foods not included in the experimental design.

The study design included an initial 28-day period during which all subjects consumed a saturated fat-rich diet (SAT diet) with 15% of energy as protein, 47% as carbohydrate, and 38% as fat (20% saturated fat [SFA], 12% monounsaturated fatty acids [MUFA] and 6% polyunsaturated fatty acids [PUFA]). The second diet lasted 28 days, and all subjects consumed a National Cholesterol Education Program type I diet (NCEP-I diet) (27) containing 15% of energy as protein, 57% as carbohydrate, and 28% as fat (10% SFA, 12% MUFA, and 6% PUFA). The third diet also lasted for 28 days, and all subjects consumed a MUFA-rich diet (MUFA diet) with 15% of energy as protein, 47% as carbohydrate, and 38% as fat (10% SFA, 22% MUFA, and 6% PUFA). Dietary cholesterol was maintained constant and the mean intake was 115 mg/1000 kcal over the three periods. The mean total energy intake per day throughout the study was 10.2 MJ. This study was approved by the Human Investigation Review Committee at the Reina Sofia University Hospital.

The composition of the experimental diets was calculated using the United States Department of Agriculture food tables (28) and Spanish food composition tables for local foodstuffs (29). Fourteen menus, prepared with regular solid foods, were rotated during the experimental period. We used virgin olive oil for cooking and salad dressing during the high MUFA period and palm oil and butter for the SAT diet. Lunch and dinner were consumed in the hospital kitchen. Breakfast and an afternoon snack were prepared by each individual with the recommended food products according to our instructions. Duplicate samples from each menu were collected, homogenized, and stored at −80°C. Protein, fat, and carbohydrate content of the diet were analyzed with standard methods. Dietary compliance was verified by analyzing the fatty acids in the LDL cholesteryl esters at the end of each dietary period.

Lipid analysis

Venous blood was collected in edetic acid-containing tubes from subjects fasted for a minimum of 12 h at the end of each of the three dietary periods. Plasma was obtained by low-speed centrifugation at 4°C within 1 h of venous puncture. Cholesterol and triglycerides were assayed by enzymatic procedures (30, 31). HDL-C was measured after precipitation of apoB-containing lipoproteins with phosphotungstic acid (32). LDL-C level was calculated from the total cholesterol, triglyceride, and HDL-C values using the Friedewald formula (33). ApoA-I and apoB concentrations were determined by turbidimetric methods (34). To reduce interassay variation, plasma and lipoprotein fractions were stored at −80°C and analyzed at the end of the study in triplicate.

LCAT activity was determined by the method described by Manabe et al. (35) and involved enzymatic determination of the decrease in free cholesterol after incubation of the serum with liposomes. Reagents were supplied by Boehringer Mannheim (Mannheim, Germany). This method measures the activity of the mass of LCAT present in plasma against an artificial substrate and does not detect differences in LCAT activity due to changes in the composition of the natural substrate.

Plasma CETP was measured by solid phase RIA using TP-2, and an anti-human CETP monoclonal antibody (from Dr. Ross Milne), and recombinant human CETP (from Dr. Alan Tall) as a standard (36). The interassay

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CV was ± 6%. Samples were measured in duplicate. We have previously demonstrated a close correlation between plasma CETP mass and in vivo isotopic transfer activity in normal subjects ($r = 0.86$) and hyperlipoproteinemic subjects ($r = 0.72$) (37).

Plasma apoA-IV levels were determined by ELISA. Polystyrene microtiter plates (Nunc Immunoplate I) were coated with affinity-purified polyclonal apoA-IV antibody (10 μg/ml) in PBS 0.1 M (pH 7.4), 100 μL/well. The plates were covered with acetate plate sealers (ICN) and incubated overnight at room temperature. The next day the solution containing the unbound antibody was removed, and the remaining binding sites in the plate were blocked by 0.5% bovine serum albumin (RIA grande BSA, Sigma) and 0.1% NaN_3 in PBS (1 h incubation). Plates were then washed 3 times with PBS containing 0.5% Tween-20 (PBST). Control and plasma samples were diluted (1:5000) in PBS-BSA. Two-fold serial dilutions were performed for the plasma standard (standard curve 333.3 ng/mL to 10.4 ng/mL). Controls were prepared in the laboratory by pooling plasma from different individuals. Multiple aliquots were stored at −70°C. Controls were calibrated against the first standard determined by amino acid analysis. Aliquots (100 μL) of standards, and control and plasma samples were added to designated wells in the microtiter plate after prior dilution and were thoroughly mixed before addition. Controls and test samples were run in duplicate. After 2 h incubation at 37°C, the contents of the plate were discarded and the plate was washed 3 times with PBST. The goat-immunopurified apoA-IV antibody conjugated to peroxidase was diluted in PBS-BSA to 1:5000 and 100 μL was added to each well. The plate was sealed and incubated at 37°C for 2 h. After this incubation, the plate was washed 5 times with PBST. The substrate for the enzymatic color reaction was 4 mg *o*-phenylene diamine (OPD) and 8 μL H_2O_2 in 12.5 mL 0.1 M citrate buffer. This solution was added to each well (100 μL) and incubated for 30 min at room temperature; it was then read at 410 nm on a microtiter plate reader (Dynatech MR 600).

DNA amplifications and genotyping of apolipoproteins A-IV and E

DNA was extracted from 10 mL of EDTA-containing blood. ApoA-IV sequences were amplified from DNA samples using PCR. The forward primer used for restriction isotyping of apoA-IV 360Gln and 360His was 5′-GCCCTGGTGCAGCAGATGGAACAGCTCAGG-3′ and the reverse primer with a mismatch (underlined) was 5′-CATCTGCACCTGCTCCTGCTGCTGCTCCAG-3′, according to the method previously described by Hixson and Powers (14). In addition to the buffer described by the supplier of Taq polymerase (Promega, Madison, WI), each amplification reaction contained 0.5 μg of DNA, 1 μmol/L of each primer, 200 μmol/L of dNTP, 2.5 U of Taq polymerase, and 10% dimethyl sulfoxide in a final volume of 50 μL. DNA was denatured at 95°C for 5 min followed by 30 cycles of amplification by primer annealing (65°C for 1 min), extension (70°C for 2 min), and denaturation (95°C for 1 min).

After amplification, one 10-μL sample of the PCR product was used for digestion with 5 units of the restriction enzyme PvuII (Promega, Madison, WI), to distinguish the coding sequences for 360Gln and 360His (14). The enzyme was added directly to the reaction mixture for digestion (>3 h at 37°C). Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel for 3 h under constant current (45 mA). Bands were visualized by silver staining. Samples containing the 360His mutation were amplified a second time to verify the genotype.

ApoE polymorphism was determined by restriction enzyme analysis of DNA amplified by PCR as previously described (38). Briefly, amplification of a 266 bp region of the fourth exon of the apoE was done by PCR with 250 ng of genomic DNA, 0.2 μmol of each oligonucleotide primer (E1, 5′-GAACAACTGACCCCGGTGGCGGAG-3′ and E2, 5′-TCGCGGGCCCCGGCCTGGTACACTGCCA-3′) and 10% dimethylsulfoxide in 50 μL. DNA was denatured at 95°C for 5 min followed by 30 cycles of denaturation at 96°C for 1 min, annealing at 63°C for 1.5 min, and extension at 72°C for 2 min. Twenty μL of the PCR product was digested with 10 units of restriction enzyme CfoI (Promega, Madison, WI) in a total volume of 35 μL. Digested DNA was separated by electrophoresis on an 8% nondenaturing polyacrylamide gel at 150 V for 2 h. Bands were visualized by silver staining.

Statistical analysis

Statistical analyses were carried out using the CSS statistical package (Statsoft, Inc. Tulsa, OK). ANOVA for repeated measures was used to analyze the effects of the apoA-IV genetic variation on plasma lipid levels for each dietary phase. When statistically significant effects were demonstrated, the Tukey's post-hoc test was used to identify group differences. A value of $P < 0.05$ was considered significant. All continuous variables, except for triglycerides, were normally distributed as assessed by the Kolmogorov-Smirnov test. Triglycerides were logarithmically transformed to achieve approximately normal distribution and statistical tests were done on the transformed values. Simple correlation analysis was also applied to results.

We used HDL-C and apoA-I responsiveness as dependent variables and did stepwise multiple regression to identify other concomitant variables. The independent variables included apoA-IV-360Gln/His and apoE genotypes, BMI, apoA-IV levels, LCAT activity, CETP level,

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TABLE 1. Baseline characteristics of the participants according to genotype (mean ± SD)

Variable	360Gln/Gln	360Gln/His	P Value[a]
Number	33	8	
Age (years)	20.6 ± 2	22 ± 2	0.081
BMI (kg/m^2)	24.6 ± 2.9	24.1 ± 2.9	0.675
Cholesterol (mg/dL)	157 ± 28	155 ± 24	0.853
Triglycerides (mg/dL)	91 ± 52	86 ± 26	0.781
LDL-C (mg/dL)	93 ± 26	97 ± 24	0.674
HDL-C (mg/dL)	46 ± 9	40 ± 12	0.179
ApoA-I (mg/dL)	114 ± 17	103 ± 26	0.169
ApoB (mg/dL)	58 ± 14	55 ± 12	0.556

[a]Student's t-test.

and basal total cholesterol and triglyceride values. The apoA-IV genotypes were divided into two classes and apoE genotype into four classes for analysis.

RESULTS

Table 1 shows basal anthropometric and plasma lipid characteristics for subjects participating in the study. No differences were observed for any of the variables between subjects carrying the His allele and those subjects homozygous for the Gln allele.

Dietary composition is recorded in **Table 2.** Dietary compliance was excellent as confirmed by the absence of significant events from the participants' diaries and the analysis of the composition of plasma cholesteryl esters (**Table 3**). Significant enrichment was observed in palmitic acid during the SAT diet. There was also a significant enrichment in oleic acid during the MUFA diet compared to the NCEP-I diet.

TABLE 2. Mean daily intake during each experimental diet period

	SAT Diet	NCEP-I Diet	MUFA Diet
Protein (% of energy intake)			
Calculated	15	15	15
Analyzed	18.1	17.6	17.5
Fat (% of energy intake)			
Saturated			
Calculated	20	10	10
Analyzed	22.6	9.2	9.2
Monounsaturated			
Calculated	12	12	22
Analyzed	10.1	13.5	24.4
Polyunsaturated			
Calculated	6	6	6
Analyzed	5	5.2	4.8
Carbohydrates (% of energy intake)			
Calculated	47	57	47
Analyzed	44.2	54.5	44.1
Cholesterol (mg/d)			
Calculated	115	115	115
Analyzed	112	113	117
Energy (MJ)	10.2	10.2	10.2

TABLE 3. Fatty acid composition of LDL-cholesteryl esters during each diet phase (mean ± SD)

Fatty Acid	SAT Diet	NCEP-I Diet	MUFA Diet
16:0	26.9 ± 1.4[a]	19.3 ± 3.9	15.2 ± 0.4
16:1	2.1 ± 0.9	2.3 ± 0.3	1.7 ± 0.2
18:0	3 ± 1.1	2.3 ± 0.8	2.5 ± 0.4
18:1	46.7 ± 4.4	38.3 ± 9	50.3 ± 4.7[b]
18:2	18.9 ± 3.6	34.9 ± 16	29.9 ± 4.8

[a]Significantly different from NECP-I ($P < 0.004$) and MUFA ($P < 0.0004$) diets (ANOVA).
[b]Significantly different from NCEP-I diet ($P < 0.03$).

Table 4 shows the plasma lipid levels after the different diets. Dietary changes were associated with significant changes in all plasma lipids except triglycerides. Genotype was found to have a significant effect on dietary-induced changes in HDL-C plasma levels, and caused changes of border-line significance in apoA-I levels.

In homozygotes for the 360Gln allele, replacement of saturated fatty acids by carbohydrates or monounsaturated fats resulted in a decrease in total cholesterol levels (NCEP-I: −20 mg/dL, $P < 0.0002$; MUFA: −19 mg/dL, $P < 0.0002$), LDL-C (NCEP-I: −18 mg/dL, $P < 0.0002$; MUFA: −16 mg/dL, $P < 0.0002$), apoA-I (NCEP-I: −8 mg/dL, $P < 0.002$; MUFA: −6 mg/dL, $P < 0.03$), and apoB (NCEP-I: −11 mg/dL, $P < 0.0002$; MUFA: −9 mg/dL, $P < 0.0002$). In carriers of the 360His allele, HDL-C (−10 mg/dL, $P < 0.0004$), apoA-I (−19 mg/dL, $P < 0.0003$) and total cholesterol levels (−27 mg/dL, $P < 0.0002$) all decreased when subjects were switched from the SAT to the NCEP-I diet. With the latter diet, HDL-C levels of these subjects were lower than in subjects homozygous for the 360Gln allele (37 vs. 44 mg/dL, $P < 0.007$). In the group with the 360His allele, consumption of the MUFA diet significantly increased the HDL-C in comparison to the NCEP-I diet (9 mg/dL, $P < 0.003$), and produced a decrease in total cholesterol (−22 mg/dL, $P < 0.002$) and LDL-C levels (−18 mg/dL, $P < 0.03$) in comparison to the SAT diet.

Figure 1 shows changes in HDL-C and apoA-I levels as absolute values on the different diets according to the genotype. Compared with homozygotes for the 360Gln allele, carriers of the 360His allele presented significantly greater decreases in HDL-C ($P < 0.004$) and apoA-I ($P < 0.04$) levels after the NCEP-I diet compared to the SAT diet. Replacement of carbohydrates by monounsaturated fats brought about a greater increase in HDL-C ($P < 0.004$) and apoA-I levels ($P < 0.04$) in subjects carrying the 360His allele than in ho-

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TABLE 4. Lipid and apolipoproteins (apo) levels (mg/dL) after the different diets with respect to the apoA-IV–360Gln/His genotype (mean ± SD)

Genotype	n	Diet	HDL-C	ApoA-I	Total Cholesterol	LDL-C	Triglycerides	ApoB
Gln/Gln	33	SAT	45 ± 9	114 ± 14	164 ± 25	101 ± 25	90 ± 39	61 ± 15
		NCEP-I	44 ± 9	106 ± 13	144 ± 24	83 ± 22	87 ± 40	50 ± 14
		MUFA	45 ± 10	108 ± 14	145 ± 22	85 ± 23	76 ± 27	52 ± 12
Gln/His	8	SAT	47 ± 19	117 ± 23	164 ± 26	98 ± 32	95 ± 29	54 ± 12
		NCEP-I	37 ± 9	98 ± 9	137 ± 16	83 ± 18	85 ± 22	46 ± 8
		MUFA	46 ± 17	107 ± 17	142 ± 23	80 ± 26	82 ± 28	47 ± 10
P value[a]		Genotype	0.751	0.792	0.698	0.756	0.804	0.281
		Diet	0.000007	0.000001	0.000001	0.000001	0.095	0.000001
		Interaction	0.025	0.055	0.472	0.796	0.792	0.635

[a]ANOVA for repeated measures.

mozygotes for the 360Gln allele. No differences were observed between genotypes in changes in plasma lipid and apopoprotein levels after the MUFA diet compared to the SAT diet.

Table 5 shows LCAT activity, and CETP and apoA-IV plasma levels at the end of each diet period. As compared to the levels measured during the SAT diet period, the NCEP-I diet period resulted in significant CETP lowering in both groups of subjects (Gln/Gln: −0.24 μg/mL, $P < 0.0005$; Gln/His: −0.33 μg/mL, $P < 0.025$). The MUFA diet elicited a similar decrease in Gln/Gln (−0.22 μg/mL) and Gln/His (−0.23 μg/mL) subjects; however, the effect was statistically significant only in Gln/Gln subjects ($P < 0.0005$) due to the lower number of heterozygotes. LCAT activity and apoA-IV levels were not affected by genotype or diet. However, changes in apoA-IV plasma levels after the MUFA diet compared to the NCEP-I diet were significantly correlated with changes in HDL-C between both diets ($r = 0.4126$, $P < 0.017$). No correlation was observed in the changes in both parameters between the SAT and the NCEP-I diets.

In our study population, multiple regression analysis revealed that the 360His mutation predicted the HDL-C response (19%, $P < 0.01$) when subjects were switched from the SAT diet to the NCEP-I diet and also

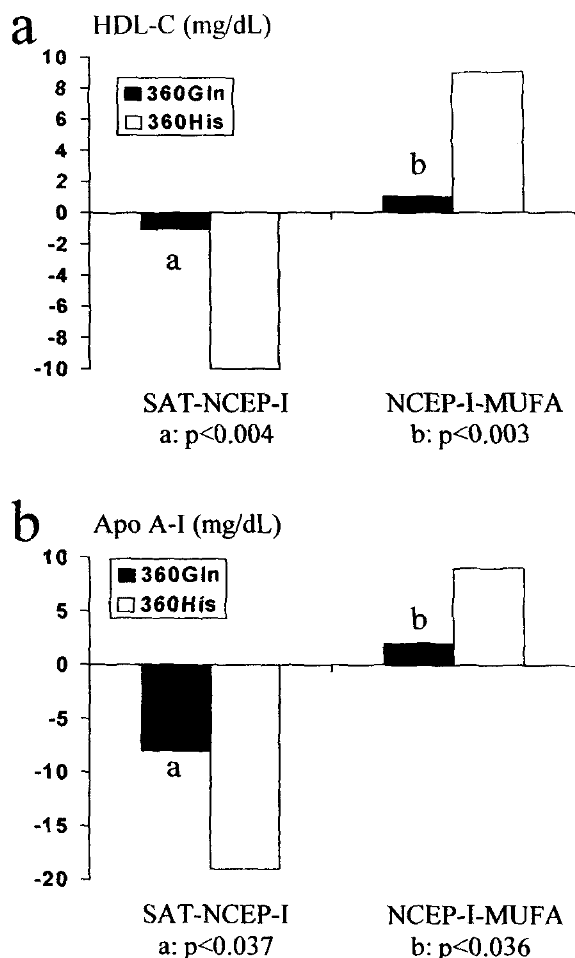

Fig. 1. Mean changes in HDL-C (a) and apoA-I (b) levels among the different diets in apoA-IV 360Gln/His genotypes.

TABLE 5. Analysis of LCAT activity (nmol of cholesterol esterified/h/mL of plasma), levels of CETP (μg/mL) and apoA-IV plasma levels (mg/dL) at the end of each dietary period according to the genotype

	LCAT	CETP	A-IV
360Gln/Gln			
SAT	480 ± 75	1.95 ± 0.44	17.8 ± 2.6
NCEP-I	489 ± 79	1.71 ± 0.37	18 ± 3.1
MUFA	495 ± 80	1.73 ± 0.37	17.6 ± 2.7
360Gln/His			
SAT	475 ± 80	2.19 ± 0.59	17.8 ± 1.5
NCEP-I	479 ± 82	1.86 ± 0.57	15.8 ± 2.3
MUFA	482 ± 75	1.96 ± 0.63	18.3 ± 1.9
P value[a]			
Genotype	0.489	0.213	0.484
Diet	0.402	0.00002	0.331
Interaction	0.647	0.589	0.183

[a]ANOVA for repeated measures.

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the HDL-C response (20%, $P < 0.009$) when switching from the NCEP-I diet to the MUFA diet. The CETP plasma level also predicted the HDL-C response when individuals were switched from the SAT to the NCEP-I diet (10%, $P < 0.047$) and from the NCEP-I diet to the MUFA diet (13%, $P < 0.02$).

DISCUSSION

Our data suggest that the 360His allele in apoA-IV is a factor affecting the HDL-C response to dietary fat. No differences were observed in plasma HDL-C between Gln/Gln and Gln/His subjects when they were consuming high fat diets. Using the equations reported by Mensink and Katan (39) in our particular experimental design, there should be an expected decrease in HDL-C of 4.7 mg/dL when saturated fatty acids are replaced by carbohydrates and an increase of 3.4 mg/dL when carbohydrates are replaced by MUFA. In subjects homozygous for the 360Gln allele, the changes were lower than expected (−1 mg/dL with the NCEP-I diet and 1 mg/dL with the MUFA diet), whereas in carriers of the 360His allele the observed changes were greater than expected: a 10 mg/dL decrease after switching from SAT to NCEP-I, and a 9 mg/dL increase after the switch from NCEP-I to MUFA. Analysis of these changes using stepwise multiple regression analysis demonstrated that the apoA-IV 360 variant was a significant independent predictor of HDL-C dietary response in this group of subjects.

In our study, in agreement with some previous reports (15–21), we did not find significant differences between basal levels of HDL-C associated with this genetic variant. In contrast, other authors have observed a significant elevation in HDL-C levels associated with the His allele (22, 23, 40). Thus, in Austrian (22) and Icelandic (23) populations, Menzel et al. have reported elevated HDL-C levels for carriers of the His allele as compared to homozygotes for the Gln allele. Similarly, in a study on dietary intervention conducted on a North American population, McCombs et al. (40) recorded similar results from analysis of the plasma lipids of participants before this intervention. Because, according to our results, plasma HDL-C levels in carriers of the 360His mutation are significantly affected by dietary fat content, the differences observed by the authors could be due to dietary differences of the populations studied. It should be noted that in most of these studies (15, 17, 19, 20, 22–24, 40) the presence of the His allele was determined by isoelectric focusing and immunoblotting; therefore, mutations other than the 360His but with similar isoelectric behavior could have been misclassified. However, this is unlikely given the much higher frequency of 360His mutation compared to the 362–365 mutation, which has a similar isoelectric point. In contrast with our results, Mata et al. (41) observed that women with the isoform A-IV-2 presented higher HDL-C levels than carriers of apoA-IV-I after consumption of both a diet rich in fat plus cholesterol and an NCEP-I type diet. Nevertheless, they did not observe these findings in the male population. This could possibly be explained by a phenomenon that was described in another study which demonstrated that sex could modify the effect of this polymorphism on plasma lipid levels (17).

Two recent studies suggest that carriers of the A-IV-2 allele could metabolize dietary fats and cholesterol in a different way than homozygotes for the A-IV-1 allele. In the previously cited work by Mata et al. (41), the decrease in LDL-C in male carriers of the A-IV-2 isoform, when these were switched from a typically American diet rich in saturated fats and cholesterol to an NCEP-I type diet low in fats and cholesterol, was significantly lower than that observed in homozygotes for the A-IV-1 allele. Similarly, McCombs et al. (40), showed that subjects with the apoA-IV-2 allele presented a lower increase in plasma LDL and total cholesterol after switching from a low cholesterol to a cholesterol-rich diet. In our work, the lack of difference in LDL-C response compared to individuals with apoA-IV360Gln could be due to the fact that determination of the isoforms in both studies was carried out by isoelectric focusing and immunoblotting, and possibly, mutations other than 360His with a similar isoelectric point could have been considered as A-IV-2 mutations, such as the deletion of a glutamic acid and three glutamines described by Hixson and Powers (14). Moreover, in the work by Mata (41), dietary design consisted in modifying fat and cholesterol content, and in McCombs' work (40) only cholesterol content was changed, whereas in our study dietary cholesterol was maintained constant. Our results, therefore, could suggest that the varying response in LDL-C levels observed in these studies (40, 41) reflects a difference in the action of dietary cholesterol, and not dietary fats, in homozygotes for the A-IV-1 isoform and carriers of A-IV-2.

We do not know the mechanism responsible for the greater increase in HDL-C in carriers of the 360His mutation. The apoA-IV gene is closely linked to the apoA-I gene (42), and it is conceivable that the 360His mutation could be a marker for an apoA-I mutation(s) that, in turn, could be responsible for the observed effects on HDL metabolism.

LCAT catalyzes the formation of cholesteryl esters in HDL and CETP is involved in the transport of these esters from HDL to other lipoproteins (43). In vitro, apoA-IV is capable of activating LCAT (16) and modulating the CETP-mediated cholesteryl ester transfer (6). We did not observe any differences in the CETP levels

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or in LCAT activity in carriers of the 360His mutation after the different dietary periods, suggesting that the mechanism involved in the greater increase in HDL-C of these individuals is not due to a difference in the activity of these enzymes. Neither did we find any differences in the apoA-IV plasma levels after the different dietary periods in the two genotypes. The apoA-IV plasma levels, however, did follow a trend similar to that of HDL-C in individuals with the 360His mutation, with a decrease (−11%) on switching from the SAT to the NCEP-I diet and an increase (+12.5%) when the carbohydrates were replaced by monounsaturated fats, but these changes did not reach significance at $P < 0.05$. In contrast, in the individuals homozygous for the 360Gln allele, apoA-IV levels were not affected by dietary changes. It is possible that the increased change in apoA-IV plasma levels with diet in subjects with the 360His allele brings about the greater increase in HDL-C with fat-rich diets. In fact, when we applied simple regression analysis to the results, changes in the apoA-IV levels after the MUFA diet compared to the NCEP-I diet were correlated with changes in HDL-C between these diets.

Regardless of the mechanism involved, our results indicate that the presence of the 360His allele of apoA-IV is responsible, at least partially, for the inter-individual variability in HDL-C response to dietary fats, with subjects who have this allele presenting a greater increase in HDL-C levels after consuming fat-rich diets. Moreover, these subjects will have a more favorable lipid profile when consuming a diet rich in monounsaturated fats than on a diet rich in carbohydrates.

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