

# Influence of apoA-V gene variants on postprandial triglyceride metabolism: impact of gender<sup>§</sup>

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**Abstract** Although apolipoprotein A-V (apoA-V) polymorphisms have been consistently associated with fasting triglyceride (TG) levels, their impact on postprandial lipemia remains relatively unknown. In this study, we investigate the impact of two common apoA-V polymorphisms (–1131 T>C and S19W) and apoA-V haplotypes on fasting and postprandial lipid metabolism in adults in the United Kingdom (n = 259). Compared with the wild-type TT, apoA-V –1131 TC heterozygotes had 15% ( $P = 0.057$ ) and 21% ( $P = 0.002$ ) higher fasting TG and postprandial TG area under the curve (AUC), respectively. Significant ( $P = 0.038$ ) and nearly significant ( $P = 0.057$ ) gender  $\times$  genotype interactions were observed for fasting TG and TG AUC, with a greater impact of genotype in males. Lower HDL-cholesterol was associated with the rare TC genotype ( $P = 0.047$ ). Significant linkage disequilibrium was found between the apoA-V –1131 T>C and the apoC-III 3238 C>G variants, with univariate analysis indicating an impact of this apoC-III single nucleotide polymorphism (SNP) on TG AUC ( $P = 0.015$ ). However, in linear regression analysis, a significant independent association with TG AUC ( $P = 0.007$ ) was only evident for the apoA-V –1131 T>C SNP, indicating a greater relative importance of the apoA-V genotype.—Olano-Martin, E., E. C. Abraham, R. Gill-Garrison, A. M. Valdes, K. Grimaldi, F. Tang, K. G. Jackson, C. M. Williams, and A. M. Minihane. **Influence of apoA-V gene variants on postprandial triglyceride metabolism: impact of gender.** *J. Lipid Res.* 2008. 49: 945–953.

**Supplementary key words** polymorphism • apolipoprotein A-V • apoA1/C3/A4/A5 gene locus • postprandial lipemia

Since its discovery in 2001 (1), a number of key roles of apolipoprotein A-V (apoA-V) in lipoprotein, and in particular triglyceride-rich lipoprotein (TRL) metabolism have been described (2–6). Evidence for its role in TRL metabolism is provided by studies in apoA-V knockout or over-

expressing rodents, in which 3- to 4-fold higher and lower plasma triglyceride (TG) levels were observed, respectively (7). Furthermore, a number of publications reporting on the associations between polymorphisms in the gene locus and fasting TG levels (1, 8) support the importance of this newly defined apolipoprotein in whole body TRL handling.

The apoA-V gene is located 27 kb distal to apoA-IV in the highly polymorphic apoA1/C3/A4/A5 gene cluster located on chromosome 11q23. Although reports are not fully consistent (9), significant associations between gene variants of the apoA-V gene locus and cardiovascular disease (CVD) and the metabolic syndrome/diabetes have been demonstrated repeatedly (10–15). Polymorphisms in the apoA-V site have also been associated with familial combined hyperlipidemia (FCH), with Ribalta and coworkers (16) suggesting that the apoA-V genotype may be responsible for 30% of the variation of TG levels in FCH families, an association that has been observed in subsequent studies (17–20).

Common single nucleotide polymorphisms (SNPs) in the apoA-V gene, in particular the apoA-V –1131 T>C in the promoter region and the apoA-V S19W (56 C>T) coding region variant (which results in a serine-to-tryptophan amino acid change in the mature protein), have been associated with increased CVD risk and fasting TG levels. These two variants, which describe the apoA-V\*1, apoA-V\*2, and apoA-V\*3 haplotypes (21), have been shown to result in up to 70% higher fasting TG (8, 13, 22–27).

In addition to fasting TG, an exaggerated postprandial lipemia is recognized as an independent predictor of CVD, associated with obesity and a loss of insulin sensitivity (28–31). Although the impact of apoA-V genotype on fasting TG has been relatively widely reported, to the best of our knowledge only three previous studies have investigated the impact of common apoA-V gene variants on

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postprandial lipemia, with two conducted in Korean male cohorts and one in young adult Caucasian males (European Atherosclerosis Research Study 2) (32–34). Here, we report on the impact of the –1131 T>C and S19W polymorphisms on postprandial TG metabolism in healthy males and females in the United Kingdom, with the data indicating that the impact of genotype may be gender-specific.

Given the reported association between other gene variants in the apoA-I/C3/A4/A5 gene cluster and fasting TG levels (26, 27, 35–37), the individual and interactive impact of other SNPs in this locus on postprandial TG metabolism were also considered.

## SUBJECTS AND METHODS

### Subjects

The participants included in the current analysis were taken from four individual studies designed to investigate the impact of chronic dietary fat manipulation on postprandial lipid (TG and NEFAs), glucose, and insulin metabolism. Here, we report on the impact of genotype on fasting and postprandial lipid responses, using the baseline data from 259 participants. At the time of the study, all participants were following their habitual diet and had not commenced the relevant chronic intervention study. All individuals were recruited using identical inclusion/exclusion criteria, and all underwent the same sequential meal postprandial protocol.

Healthy adults in the United Kingdom aged 20–70 years, with fasting total cholesterol between 4.6 and 8.0 mmol/l and TG between 1.0 to 4.0 mmol/l, were recruited by a variety of means, including e-mailing staff at the university with a general description of the study, advertising in the local media, and through a database held at the Department of Clinical Pathology, Royal Berkshire Hospital, Reading, UK. Those interested in taking part were asked to contact the Hugh Sinclair Unit of Human Nutrition to complete a health and lifestyle questionnaire and to provide a screening blood sample. Exclusion criteria for participation in the study included the following: evidence of CVD, including angina; diagnosed diabetes or fasting glucose > 6.5 mmol/l; liver or other endocrine dysfunction; pregnancy or lactation; smoking of >15 cigarettes per day; exercising strenuously more than three times per week; body mass index (BMI) of <20 or >32 kg/m<sup>2</sup>; and hemoglobin < 130 g/l in men or 120 g/l in women. Individuals who were prescribed hyperlipidemic or anti-inflammatory medication, who took fatty acid or antioxidant supplements on a regular basis, who consumed sterol/stanol-containing spreads, or who consumed more than one portion of oily fish per week were excluded. The studies were approved by the University of Reading Ethics and Research Committee and the West Berkshire Health Authority Ethics Committees, and each volunteer gave written informed consent before participating.

### Postprandial protocol

The day before their postprandial assessment, participants were asked to refrain from alcohol or organized exercise regimens and were provided with a relatively low-fat (<10 g of fat) evening meal to standardize short-term fat intake. After a 12 h overnight fast, an indwelling cannula was inserted into the antecubital vein of the forearm and a fasting blood sample was taken. After a standard test breakfast (0 min) and lunch (330 min), blood samples were taken at 0, 60, 120, 180, 240, 300, 330, 360, 390, 420, and 480 min after breakfast for plasma TG and NEFA analysis. The nutritional content of the test breakfast was

3.9 MJ of energy, 111 g of carbohydrate, 19 g of protein, and 49 g of fat, comprising 29.6 g of saturated fatty acids (SFAs), 12.2 g of MUFAs, 1.6 g of PUFAs, and 2.5 g of *trans* fatty acids. The nutritional content of the test lunch was 2.3 MJ of energy, 63 g of carbohydrate, 15 g of protein, and 29 g of fat, which contained 14.3 g of SFAs, 7.1 g of MUFAs, 3.0 g of PUFAs, and 2.9 g of *trans* fatty acids. This nutritional information was derived using food nutrient data from McCance and Widdowson's Food Composition Tables, supplemented with a food fatty acid content database (Foodbase2000, London, UK).

### Blood handling

Baseline and postprandial venous blood samples were collected into 3 × 10 ml of EDTA and 1 × 2 ml of fluoride oxalate tubes for glucose analysis. All samples, except those collected for LDL isolation (1 × 10 ml of EDTA), were centrifuged at 1,600 g for 10 min, within 1 h of collecting the blood. Plasma subsamples were stored at –20°C for postprandial TG and NEFA and for fasting total cholesterol, glucose, and insulin analysis (0 h only). In the 0 min sample, HDL-cholesterol (HDL-C) was determined by measuring cholesterol in the supernatant after precipitation of the apoB-containing lipoproteins using dextran sulfate and magnesium chloride (38). LDL-cholesterol (LDL-C) levels were computed using the Friedewald formula (39).

### Biochemical analysis

Plasma TG, total cholesterol, HDL-C, NEFA, and glucose concentrations were quantified using an automated clinical chemistry analyzer (Instrumentation Laboratory, Ltd., Warrington, UK) using enzymatic colorimetric kits [Instrumentation Laboratory, Ltd. for TG, cholesterol, and glucose; Alpha Laboratories (Eastleigh, UK) for NEFA]. Insulin was assayed using a specific ELISA kit (Dako, Ltd., High Wycombe, UK). The mean intra-assay coefficients of variation for the TG, cholesterol, NEFA, glucose, and insulin assays were 2.3, 1.6, 1.5, 3.2, and 4.5%, respectively.

LDL subclasses were separated from 10 ml of EDTA blood at 0 min only by density-gradient ultracentrifugation, as described previously (40).

### Expression of postprandial data

The postprandial TG and NEFA responses were expressed as area under the curve (AUC; 0–480 min), calculated using the trapezoidal rule, or incremental area under the curve (IAUC; 0–480 min), calculated as AUC minus fasting concentrations. Because the NEFA concentrations decrease sharply in the immediate postprandial period and increase postprandially as a result of increased chylomicron and adipose tissue lipolysis, the shape of the postprandial NEFA response is complex, representing a number of metabolic events. In the data analysis, percentage NEFA suppression at 0–120 min was used as an index of insulin sensitivity with respect to fatty acid metabolism, in addition to AUC. The percentage NEFA suppression in the first 120 min largely reflects insulin-induced suppression of adipose tissue lipolysis and may be considered an index of insulin sensitivity (41).

### DNA extraction and genotyping

DNA was isolated from the buffy coat layer of 10 ml of EDTA blood using the Qiagen DNA Blood Mini Kit (Qiagen, Ltd., Crawley, UK). Allelic discrimination of the apoA-V –1131 T>C (rs 662799), S19W (rs 3135506), ApoC3 3238 C>G (SstI, S1/S2, rs5128), apoA-IV S347T (rs675), and apoA-IV Q360H (rs 5510) gene variants was conducted using TaqMan PCR technology (7300 Instrument; Applied Biosystems, Warrington, UK) and Assay-on-Demand SNP genotyping assays (Applied Biosystems).

## Data analysis and statistics

Hitagene Gene Hunting System Software (Hitachi, Dublin, Ireland) was used to investigate the pair-wise strength of linkage disequilibrium (LD) between SNPs (with the LD between two SNPs estimated using  $D'$ ) and estimated haplotype frequencies. Deviations from Hardy-Weinberg equilibrium were assessed using the exact test by the Markov chain modeling method as implemented by Genepop ([www.genepop.curtin.edu.au](http://www.genepop.curtin.edu.au)).

The ApoA-V\*1, ApoA-V\*2, and ApoA-V\*3 haplotypes were defined by the presence of the apoA-V -1131T/56C, -1131C/56C, and -1131T/56G alleles, respectively (18). Furthermore, strong LD was observed between the apoA-V -1131T>C and apoC-III 3238C>G SNPs; therefore the combined impact of these SNPs was also considered.

All biochemical outcomes are expressed as means and (SEM). The impact of genotype on fasting and postprandial (AUC and IAUC) lipid responses was determined using one-way analysis of co-variance (ANCOVA), with BMI, gender, and age as covariants. The significance of gender  $\times$  genotype, age  $\times$  genotype, and BMI  $\times$  genotype interactions was established using multiple analysis of variance (MANOVA) with gender, age, and BMI entered into the model as independent variables. Because of the relatively small group sizes, full haplotype analysis was not conducted, but the independent contributions of individual SNPs or SNP  $\times$  gender/age/BMI interactions to the lipid responses were established using stepwise regression analysis.

Statistical analysis was carried out using the S-Plus 6.1 statistical package (Insightful Corp., Seattle, WA), with  $P < 0.05$  taken as significant.

## RESULTS

A total of 262 individuals, 153 males and 109 females, underwent postprandial assessment, with complete postprandial data available for 259, which included 235 Caucasians and 24 non-Caucasians of South Asian or Afro-Caribbean origin. The mean age, BMI, and fasting plasma lipids, glucose, and insulin for the cohort as a whole and according to gender are presented in **Table 1**.

TABLE 1. Baseline characteristics of the study group as a whole and according to gender

Outcome	All (n = 262)	Males (n = 153)	Females (n = 109)	$P^a$
Age (years)	52.7 (0.7)	53.0 (0.8)	52.2 (1.1)	0.560
BMI (kg/m <sup>2</sup> )	26.2 (0.2)	27.3 (0.3)	25.4 (0.3)	<0.001
Total cholesterol (mmol/l)	5.76 (0.06)	5.90 (0.08)	5.55 (0.10)	0.007
LDL-C (mmol/l)	3.71 (0.06)	3.91 (0.08)	3.42 (0.10)	<0.001
HDL-C (mmol/l)	1.32 (0.03)	1.11 (0.02)	1.62 (0.04)	<0.001
% LDL <sub>3</sub>	52.1 (2.1)	54.6 (2.8)	47.1 (2.0)	0.110
TG (mmol/l)	1.64 (0.05)	1.97 (0.07)	1.18 (0.04)	<0.001
NEFA ( $\mu$ mol/l)	512 (11)	499 (14.7)	531 (18)	0.160
Glucose (mmol/l)	5.16 (0.04)	5.34 (0.05)	4.89 (0.05)	<0.001
Insulin (pmol/l)	48.6 (1.9)	52.5 (2.5)	36.8 (2.4)	0.003

Values are means (SEM). BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; % LDL<sub>3</sub>, percentage of the total LDL as the small dense LDL<sub>3</sub> fraction; TG, triglyceride. For % LDL<sub>3</sub>, NEFA, and insulin, data were only available for n = 97 (29 females and 67 males), n = 255 (106 females and 149 males), and n = 167 (42 females and 125 males), respectively.

<sup>a</sup>Intergender differences tested using one-way ANOVA.

The genotype distribution of the five polymorphisms included in the analysis did not deviate significantly from Hardy-Weinberg equilibrium ( $P = 0.3806$ – $0.9986$ ). Rare allele frequencies of C = 0.09, G = 0.05, T = 0.19, A = 0.08, and G = 0.23 were evident for the apoA-V -1131T>C, apoA-V S19W, apoA-IV T347S, apoA-IV Q360T, and apoC-III 3238C>G SNPs, respectively. Comparable rare allele frequencies of 0.13, 0.02, 0.13, 0.06, and 0.19 were evident in the non-Caucasian subgroup relative to the group as a whole.

To determine the degree of LD in our study sample, standardized LD coefficients ( $D'$ ) was calculated for all pairs of SNPs. The LDs between the individual variants were comparable to those reported previously (see supplementary Figure I), with significant LD between the two apoA-V SNPs ( $P = 0.024$ ), the apoA-V -1131T>C and apoC-III 3238C>G variants ( $P = 0.000$ ), and the apoA-V S19W and apoA-IV T347S SNPs ( $P = 0.013$ ). No significant LD was evident between the apoA-IV and apoA-V variants.

The two polymorphic sites in the apoA-V gene resulted in three observed haplotypes, as described in **Table 2**, which had frequencies of 85.6, 9.4, and 4.8% for apoA-V\*1, apoA-V\*2, and apoA-V\*3, respectively, in our 259 unrelated participants. As the alleles are in strong LD, the apoA-V -1131C/56G haplotype is rare and was not observed in the present study. Based on these three haplotypes, four haplotype combinations were observed, with 189, 40, 25, and 3 participants having an apoA-V\*1/apoA-V\*1, apoA-V\*1/apoA-V\*2, apoA-V\*1/apoA-V\*3, and apoA-V\*2/apoA-V\*2, genotype, respectively, and no individuals presenting with the apoA-V\*2/apoA-V\*3 or apoA-V\*3/apoA-V\*3 genotype (**Table 3**).

No significant impact of apoA-V polymorphisms or haplotypes on fasting TG responses was evident, although the impact of the apoA-V -1131 T>C SNP reached borderline significance ( $P = 0.079$ ). In contrast, a significant association between the -1131T>C SNP and postprandial TG responses was evident (Table 3, **Fig. 1A**), with 20.9% ( $P = 0.002$ ) and 25.3% ( $P = 0.041$ ) higher TG AUC and TG IAUC in the heterozygote rare allele carriers (apoA-V -1131 TC) compared with the TT subgroup. This affect of the -1131T>C genotype was reflected in the haplotype combination association analysis with TG AUCs (mmol/l/480min) of 1,103 (39), 1,347 (206), 1,228 (120), and 1,425 (412) observed in the apoA-V\*1/apoA-V\*1, apoA-V\*1/apoA-V\*2, apoA-V\*1/apoA-V\*3, and apoA-V\*2/apoA-V\*2 groups, respectively (Table 3, **Fig. 2**) ( $P = 0.008$ ).

No significant impact of age on the associations between apoA-V SNPs or haplotypes and the fasting or postprandial TG responses was observed.

TABLE 2. Structures and frequencies of the three common apoA-V haplotypes

Haplotype	Frequency	-1131 T>C	56 C>G
ApoA-V*1	85.6%	T	C
ApoA-V*2	9.4%	C	C
ApoA-V*3	4.8%	T	G

ApoA-V, apolipoprotein A5 gene. The haplotype frequencies were determined using Hitagene software.

TABLE 3. Fasting and postprandial lipid concentrations according to apoA-V -1131 T>C and apoA-V S19W (56C>G) genotypes and apoA-V haplotypes

Variable	ApoA-V -1131 T>C			ApoA-V S19W (56C>G)			ApoAV Haplotype Combinations						P <sup>a</sup>
	TT	TC	CC	CC	CG	CG	A5*1/A5*1	A-V*/1/apoA-V*2	apoA-V*/1/apoA-V*3	apoA-V*2/apoA-V*2	apoA-V*2/apoA-V*3		
n	214	42	3	232	25	25	189	40	25	3	3		
Genotype (%)	82.6	16.2	1.2	90.3	9.7	9.7	73.2	16.0	9.7	1.2	1.2		
Fasting TG (mmol/l)	1.59 (0.05)	1.83 (0.16)	1.74 (0.31)	1.63 (0.05)	1.48 (0.11)	1.48 (0.11)	1.60 (0.06)	1.79 (0.16)	1.48 (0.11)	1.74 (0.31)	1.74 (0.31)	NS	
TG AUC (mmol/l/480 min)	1,118 (37)	1,352 (104)	1,426 (412)	1,151 (37)	1,228 (120)	1,228 (120)	1,103 (39)	1,347 (106)	1,228 (120)	1,426 (412)	1,426 (412)	0.008	
TG IAU (mmol/l/480 min)	326 (15)	409 (42)	352 (177)	337 (14)	368 (57)	368 (57)	320 (15)	411 (43)	368 (57)	352 (176)	352 (176)	0.072	
Fasting NEFA (μmol/l)	533 (13)	432 (22)	353 (32)	516 (12)	501 (26)	501 (26)	534 (14)	430 (23)	501 (26)	352 (32)	352 (32)	0.006	
NEFA AUC (μmol/l/480 min)	190.2 (3.5)	171.8 (5.5)	138.6 (5.4)	188.0 (3.3)	172.2 (7.7)	172.2 (7.7)	192.3 (3.9)	172.7 (5.6)	172.2 (7.7)	138.6 (5.4)	138.6 (5.4)	0.041	
% NEFA suppression	-71.3 (1.2)	-68.6 (2.9)	-73.4 (1.6)	-70.3 (1.2)	-75.7 (2.2)	-75.7 (2.2)	-70.8 (1.3)	-68.0 (2.9)	-75.7 (2.2)	-73.4 (1.6)	-73.4 (1.6)	NS	
Total cholesterol (mmol/l)	5.77 (0.07)	5.66 (0.13)	5.35 (0.54)	5.75 (0.07)	5.69 (0.24)	5.69 (0.24)	5.77 (0.08)	5.65 (0.13)	5.69 (0.24)	5.35 (0.54)	5.35 (0.54)	NS	
HDL-C (mmol/l)	1.34 (0.03)	1.25 (0.06)	1.05 (0.15)	1.32 (0.03)	1.41 (0.10)	1.41 (0.10)	1.34 (0.03)	1.26 (0.06)	1.41 (0.10)	1.05 (0.15)	1.05 (0.15)	NS	
LDL-C (mmol/l)	3.72 (0.07)	3.58 (0.13)	3.52 (0.48)	3.69 (0.06)	3.70 (0.25)	3.70 (0.25)	3.72 (0.07)	3.58 (0.13)	3.70 (0.25)	3.52 (0.48)	3.52 (0.48)	NS	
% LDL <sub>3</sub>	50.8 (1.4)	57.5 (3.3)	—	51.3 (1.3)	56.9 (4.4)	56.9 (4.4)	49.9 (1.5)	56.3 (3.3)	56.7 (4.4)	—	—	NS	

Values are means (SEM). AUC, area under the curve; IAU, incremental area under the curve; % NEFA suppression = [(NEFA 0 min - NEFA 120 min/NEFA 120 min) × 100. A5\*1, A5\*2, and A5\*3 haplotypes are defined by the presence of the apoA-V -1131T/56C, -1131C/56C, and -1131T/56G alleles, respectively.

<sup>a</sup> P is for one-way analysis of co-variance (ANCOVA), with BMI, gender, and age as covariates.

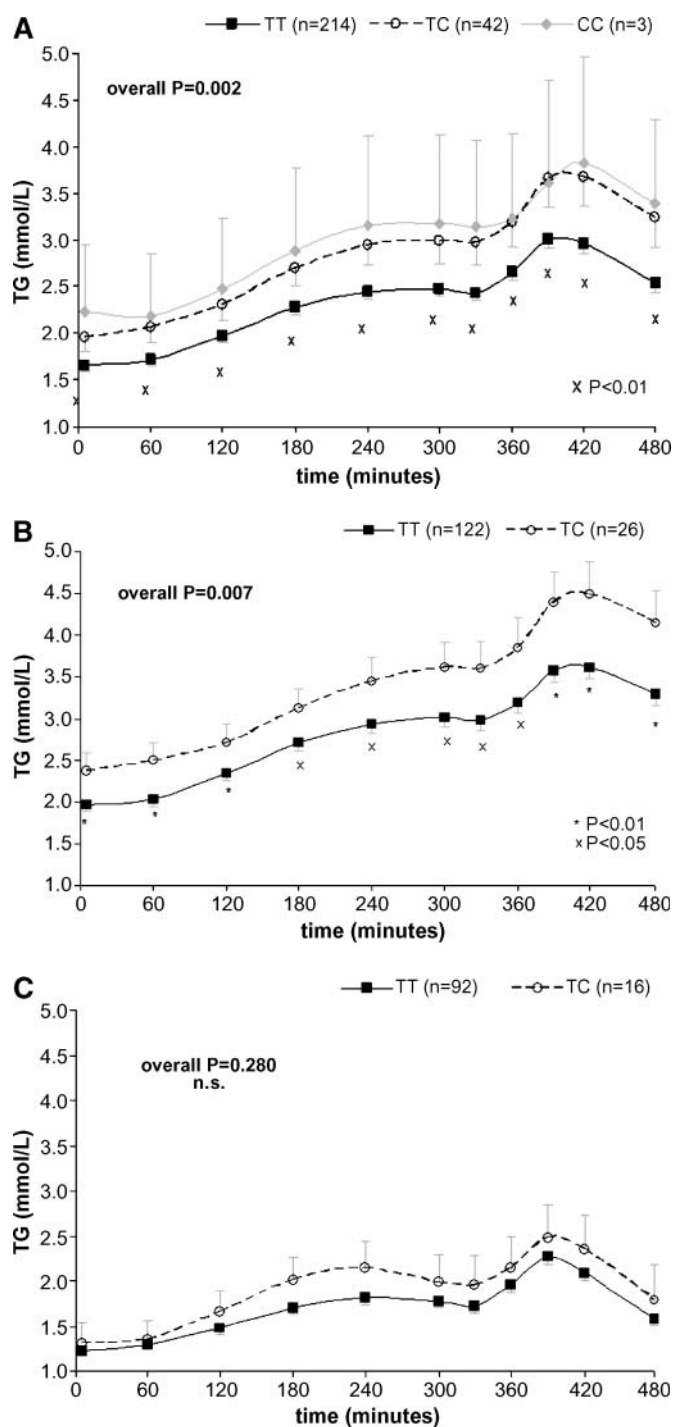
However, a significant gender × A-V -1131 T>C interaction emerged for fasting TG ( $P = 0.038$ ), with subsequent within-gender group analysis comparing TC versus TT individuals indicating a significant effect of genotype in males ( $P = 0.003$ ) but not in females ( $P = 0.962$ ). Mean TG levels of 2.22 (0.21), 1.90 (0.08), 1.13 (0.09), and 1.19 (0.05) mmol/l were evident in TC males, TT males, TC females, and TT females respectively (data not shown). Given that fasting glucose and insulin were significantly higher in the male subgroup, a subsequent ANCOVA was conducted, with glucose included as a covariate as a biomarker of insulin sensitivity. This analysis yielded a borderline significant interaction of the apoA-V -1131 T>C genotype with respect to fasting TG levels ( $P = 0.066$ ). Although the impact of gender on the associations between the apoA-V -1131 T>C polymorphism and TG AUC only reached borderline significance ( $P = 0.057$ ), there was a strong indication of a greater impact of this SNP in males relative to females. Within-gender subgroup analysis indicated a significant difference between the apoA-V TT and TC carriers only in the male cohort (25.5%;  $P = 0.007$ ), with the 9.4% intergenotype differences in TG AUC observed in females failing to reach significance ( $P = 0.280$ ) (Fig. 1B, C).

In addition to TG, apoA-V genotype influenced both fasting and postprandial NEFA levels (Table 3), with 18.9% and 9.7% lower fasting NEFA and NEFA AUC in the apoA-V TC group relative to the wild-type TT genotype. No significant effect of genotype on percentage NEFA suppression at 120 min was evident, with values of -68.6% and -71.3% observed in the TC versus TT carriers (Table 3). Furthermore, in contrast to TG, there was no significant impact of gender on genotype-NEFA associations.

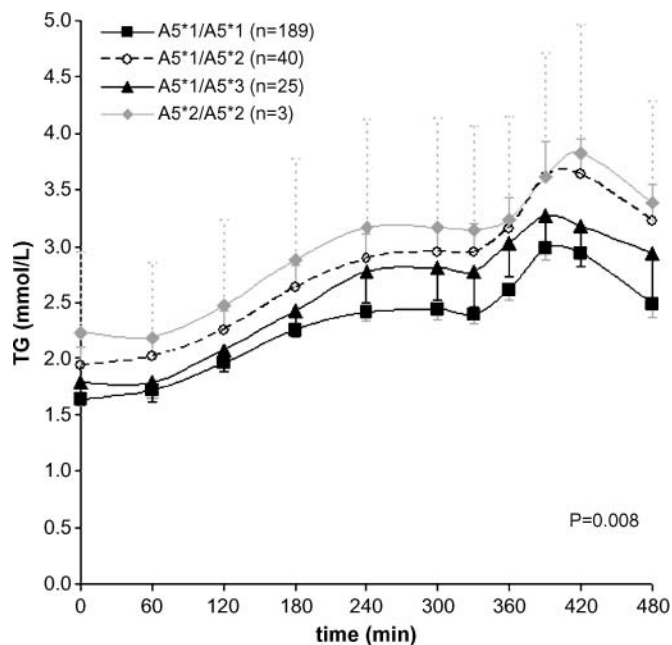
An impact of the 1131 T>C genotype on fasting HDL-C values was also observed, with circulating concentrations of 1.05 (0.15), 1.25 (0.06), and 1.34 (0.03) mmol/l evident in the CC, TC, and TT subgroups ( $P = 0.047$ ) (Table 3). No overall genotype × gender association was observed ( $P = 0.550$ ). However, there was a trend toward a greater effect of genotype in men, with the within-group TC versus TT analysis only significant in males ( $P = 0.042$ ; data not shown).

No significant impact of the apoC-III 3238C>G, apoA-IV T347S, or apoA-IV Q360H SNPs on fasting total cholesterol, HDL-C, LDL-C, percentage LDL<sub>3</sub>, or fasting or postprandial glucose, insulin, or NEFA responses was evident. Univariate analysis indicated significantly higher postprandial TG responses ( $P = 0.015$ ) in apoC-III 3238 G-carriers, with 15.0% (n = 58) and 23.2% (n = 4) higher TG AUC in CG and GG genotypes, respectively relative 10 CC homozygotes (Table 4). No age × genotype or gender × genotype interactions were evident for these apoC-III and apoA-IV SNPs.

Given the strong LD between the apoC-III -1131 T>C and the apoC-III 3238 C>G genotypes, the interactive effect of these SNPs was considered, as demonstrated in Fig. 3. A nearly significant impact of genotype was evident, using age-, gender-, and BMI-adjusted ANCOVA ( $P = 0.079$ ), with 22.5% higher TG AUC evident in -1131TC/3238CG heterozygotes compared with the wild-type 1131TT/3238CC subgroup.



**Fig. 1.** Impact of the apolipoprotein A-V (apoA-V) -1131 T>C genotype on the postprandial triglyceride (TG) response. Values are means (bars are SEM) for the group as a whole ( $n = 259$ ) (A), for males only ( $n = 148$ ) (B), and for females only ( $n = 108$ ) (C). In the males-only and females-only plots, the CC subgroup responses are omitted because of small group numbers. In A,  $P$  is for one-way analysis of co-variance (ANCOVA) with body mass index (BMI), gender, and age as covariates; in B, C,  $P$  is for one-way ANCOVA with BMI and age as covariates. For the postprandial TG responses indicated above, the gender  $\times$  genotype association as determined from the interaction term included in the ANOVA only reached borderline significance ( $P = 0.057$ ). Within-gender statistical analysis as indicated in B and C indicated a significant difference between the TT and TC genotype groups only in males.



**Fig. 2.** Impact of the apoA-V\*1 haplotype on the postprandial TG response. Values are means (bars are SEM) for the A-V\*1, A-V\*2, and A-V\*3 haplotypes defined by the presence of the apoA-V -1131T/56C, -1131C/56C, and -1131T/56G alleles, respectively.  $P$  is for one-way ANCOVA with BMI, gender, and age as covariates.

In the stepwise linear regression model, only gender  $\times$  apoA-V -1131T>C ( $P = 0.000$ ), age ( $P = 0.024$ ), BMI ( $P < 0.001$ ), apoA-V -1131T>C ( $P = 0.007$ ), and apoA-V -1131T>C  $\times$  gender ( $P = 0.038$ ) emerged as significantly associated with the TG AUC, with no significant independent association with the apoC-III 3238 C>G SNP evident.

## DISCUSSION

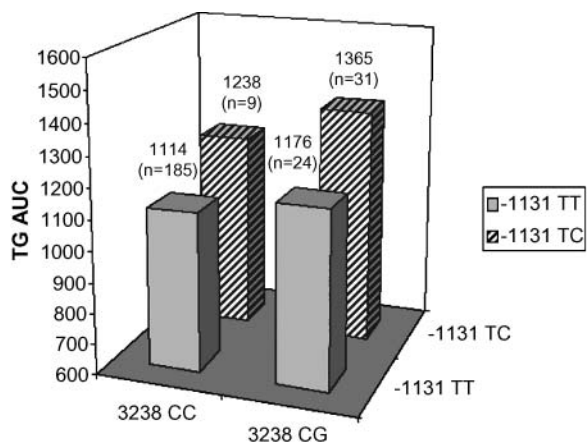
Recent evidence is suggestive that common variations of the apoA-V gene locus are significant independent pre-

**TABLE 4.** TG responses according to apoC-III 3238 C>G, apoA-IV T347S, and apoA-IV Q360H genotypes

Genotype	Fasting TG	TG AUC	TG LAUC
	mmol/l	mol/l/480 min	
<b>ApoC-III C3238G</b>			
CC ( $n = 196$ )	1.60 (0.06)	1122 (40)	332 (15)
CG ( $n = 58$ )	1.79 (0.11)	1290 (80)	365 (35)
GG ( $n = 4$ )	1.72 (0.57)	1383 (200)	470 (48)
$P^a$	NS	0.014	NS
<b>ApoA-IV T347S</b>			
TT ( $n = 168$ )	1.64 (0.06)	1140 (41)	334 (17)
TS ( $n = 59$ )	1.60 (0.09)	1180 (70)	345 (26)
SS ( $n = 10$ )	2.01 (0.46)	1376 (263)	425 (76)
$P^a$	NS	NS	NS
<b>ApoA-IV Q360H</b>			
QQ ( $n = 192$ )	1.54 (0.06)	1097 (38)	331 (16)
QH ( $n = 39$ )	1.54 (0.11)	1050 (71)	290 (36)
$P^a$	NS	NS	NS

Values are means (SEM).

<sup>a</sup>  $P$  is for one-way ANCOVA, with BMI, gender, and age as covariates.



**Fig. 3.** The interactive impact of the apoA-V -1131 T>C and apoC-III 3238 C>G genotypes on postprandial TG metabolism. TG AUC, area under the curve for the TG response (mmol/l/480 min).  $P = 0.079$  for one-way ANCOVA with age, BMI, and gender as covariates.

dictors of the risk and incidence of CVD and the metabolic syndrome (10–15, 25). For example, in case-control studies conducted in Chinese (-1131 TT vs. CC) and Hungarian (-1131 TT vs. C carriers) cohorts, adjusted odds ratios of coronary artery disease of 1.80 (95% confidence interval, 1.30–3.04) and 1.99 (95% confidence interval, 1.04–3.13) were reported (11, 12).

Based on current understanding of the role of apoA-V as a mediator of the synthesis and hydrolysis of TRL in the circulation and the association between common apoA-V SNPs and fasting TG levels (17–24, 26, 27, 37), it is likely that the increased disease risk may be attributable in part to an impact of genotype on TG metabolism. However, studies to date have largely focused on the measurement of fasting TG levels. Although the role of postprandial lipoproteins in the development of CVD is well established (29–31), and in Western societies individuals consuming multiple meals will spend an estimated 18–20 h of the day in the nonfasted postprandial state, knowledge of the impact of genotype on postprandial lipemia is distinctly lacking. Only three studies have examined the impact of apoA-V genotypes, and these studies have included only male subjects (32–34). In the European Atherosclerosis Research Study 2, which included Caucasian males (18–28 years), Martin and coworkers (34) observed significant but modestly higher postprandial TG responses in rare allele carriers (apoA-V -1131TC and apoA-V S19W) compared with homozygote wild-type genotypes. However, the association was not retained when the data were corrected for fasting TG values. In a Korean male cohort (20–50 years), exaggerated postprandial TG responses were observed in -1131 TC+CC participants compared with the TT group, with the effect largely attributable to an exaggerated TG content of the VLDL-rich fraction rather than the chylomicron-rich fraction (32). In contrast, Kim and coworkers (33) noted 62, 59, and 76% higher fasting TG, TG AUC, and chylomicron-TG AUC, respectively, in the TC versus the TT subgroups.

The results of the current study are generally consistent with previous studies, with our univariate analysis indicating 15, 21, and 25% higher fasting TG, TG AUC, and TG IAUC values, respectively, in -1131TC individuals relative to the common TT genotype. Although the kinetics of TRL secretion and clearance was not determined in the current trial, the observation that the apoA-V 1131 T>C is associated with both fasting TG levels and the postprandial incremental and total TG responses to standard meals is suggestive of an impact of genotype on both VLDL synthesis and/or postprandial TRL clearance. The possibility that both sites may be involved in determining differential TG responses to standard meals is consistent with the proposed functions of the apoA-V protein as both a regulator of VLDL synthesis and secretion and a cofactor in LPL-mediated TRL hydrolysis (2–4, 42, 43).

Some studies have suggested a major role for apoA-V in TRL clearance. A comparison of individuals with the rare apoA-V Q139X genotype (which results in a highly truncated 144 amino acid protein) with matched controls revealed severe chylomicronemia in homozygotes and heterozygotes with Q139X (44) to be associated with significant reductions in TRL lipolytic rates and postheparin LPL activities. No effect of ApoA-V genotype on VLDL apoB production rates were observed, although VLDL-TG secretion rates were not determined. ApoA-V is thought to act as a “linking” molecule that targets TRL to the LPL-proteoglycan complex at the capillary endothelium (4, 42). By binding to LPL and to endothelial proteoglycans at one site and to TRL at another, apoA-V is thought to stabilize dimeric LPL and facilitate LPL-mediated hydrolysis.

However, a role of apoA-V in VLDL synthesis and secretion has been demonstrated, with a proposed impact of the protein, on pre-VLDL trafficking from the rough endoplasmic reticulum to the Golgi apparatus and to subsequent VLDL lipid loading (2, 3, 43). These findings have not been consistently described in the literature.

In addition to apoA-V variants, a number of common polymorphisms in the ~60 kb incorporating the ApoA1/C3/A4/A5 gene cluster have been associated with alterations in both fasting and nonfasting TG metabolism. With regard to fasting TG, a number of recent studies have investigated which of the SNPs in the chromosome 11q23 DNA region are functional and which are simply in LD and serve as biomarkers for functional SNPs in the same or neighboring apolipoprotein gene (9, 26, 27, 36, 37). However, no such data are available for postprandial metabolism.

As has been observed, in the current study a complex genotype association pattern was evident across the gene cluster, with significant LD between the apoA-V -1131 T>C and the apoC-III gene variants, but no LD observed with the intervening apoA-V S19W and apoA-IV SNPs. In contrast, the apoA-V S19W locus was in significant LD with the apoA-IV T347S site. This pattern of inheritance is in close agreement with previous findings (36, 37) and is indicative, as suggested by Olivier and coworkers (36), of the occurrence of significant recombination events in the apoA-V/C3 intergenic region, with the two genes separated by a region of low LD.

The strong LD between apoA-V -1131 and apoC-III, and the recognized known association between this apoC-III variant and fasting TG levels (with an association with postprandial TG metabolism evident in the current trial), raise the possibility that the association seen between postprandial lipemia and the apoA-V variant is simply attributable to the impact of the apoC-III rare allele. A large number of studies have reported an association between common apoC-III gene variants and atherosclerosis, FCH, and fasting TG levels, in particular the apoC-III 3238C→G (S1/S2, *SstI*) polymorphism in the noncoding 3' untranslated region (26, 27, 35–37, 45).

In the current trial, there is an indication of an independent and additive effect of the two variants, with univariate analysis of the combined genotype subgroups demonstrating 6, 11, and 22% increases in TG AUC in 1131TT/3238CG, 1131TC/3238CC, and 1131TC/3238GC heterozygotes relative to the 1131TT/3238CC subgroup. However, only the apoA-V SNP remained significant in the regression analysis. These data are suggestive of a greater relative importance of the apoA-V -1131 T>C SNP compared with the apoC-III variant, consistent with the findings of Wright and coworkers (27), who examined the relative association of various apoA-V and apoC-III variants with hypertriglyceridemia.

However, the possibility cannot be discounted that although these SNPs appear to be independently associated with TG, they are acting as biomarkers for other functional SNPs elsewhere in the gene cluster. For example, a suggested candidate for the apoA-V -1131T>C is the -3A>G minor allele, which is located 3 bp upstream from the predicted start codon for apoA-V (27). This SNP is in the Kozak sequence of the gene; therefore, the variant may influence gene translation and apoA-V concentrations (7). For the apoC-III 3238G SNP, which is found in the 3' untranslated region of exon 4, no functional role has been described to date, although there has been a suggestion that this region may play a role in mRNA stability. Alternatively, it may be that the functional variant in the apoC-III locus is in fact the common apoC-III -482 C>T variant in the insulin response element in the gene promoter region (35), which is known to be in strong LD with the 3238G variant in the 3' untranslated region. Rare allele carriers are thought to be less responsive to insulin (46) and demonstrate increased expression of the apoC-III gene, thereby affecting TRL clearance rates.

An important aspect of the present findings is the observed impact of gender on genotype-phenotype responses. Interestingly, the impact of the -1131C allele on fasting and postprandial TGs was only evident in the male participants. An impact of gender on genotype-lipoprotein associations has been reported previously (47, 48) for a number of genes and specific lipid CVD risk factors. It is likely that the impact of sex steroid hormones at a number of molecular loci that regulate lipoprotein metabolism may modulate the penetrance of individual gene variants. For example, in a study examining the impact of the apoE  $\epsilon$  genotype on HDL characteristics, the authors speculated that the lack of association evident in men compared with

women is likely to be attributable to the inherently higher hepatic lipase activity in males, which may “overwhelm” the more subtle impact of the apoE genotype (48).

In the current study, 25.5% ( $P = 0.007$ ) and 9.4% ( $P = 0.280$ ) higher TG AUC was evident in TC versus TT males and females, respectively. Although the menopausal status of the female study participants was not verified, the mean age of 52 years for the female subjects indicates that at least 40–50% of the subjects were likely to have been premenopausal or perimenopausal. This is supported by the observation of significantly lower TG and higher HDL-C values in females than in males, a difference that is lost in postmenopausal women. Speculatively, it is suggested that in the premenopausal women, estrogen, with its known impact on receptor- and nonreceptor-dependent stages in lipoprotein metabolism, including TRL metabolism (49), will in part mask the effect of the apoA-V genotype on TG metabolism. Furthermore, it is possible that an impact of gender on glucose homeostasis may also be in part responsible, as the interaction between apoA-V genotype and gender only reached borderline significance when fasting glucose was included in the model as a biomarker of insulin sensitivity.

An association of the apoA-V genotype with fasting HDL-C levels was also evident, with 7% lower levels observed in -1131TC carriers. These differences in HDL-C are likely to be, in part, secondary to the genotype-associated TG differences reported above. These findings are consistent with the well-established inverse relationship between TG and HDL-C, which reflects neutral lipid exchange from TRL to HDL particles during reverse cholesterol transport (50). Although a significant gender  $\times$  genotype association was not evident for HDL-C, trends toward greater differences in males were observed.

An unexpected finding in the present study was the observation of lower fasting NEFA and lower NEFA AUC in TC carriers. Differences in NEFA concentrations have not been reported previously for this SNP; however, we observed 19% lower fasting NEFA concentrations in TC carriers, which is indicative of a significant alteration in NEFA metabolism, reflecting either decreased release from adipose tissue or greater uptake into peripheral tissues and the liver. The impact of the apoA-V 1131 T>C genotype on fasting NEFA is unlikely to reflect an altered sensitivity to the antilipolytic actions of insulin, because percentage NEFA suppression did not differ between TC and TT carriers. Given the putative role of apoA-V in promoting VLDL synthesis and secretion, a possible explanation lies in the increased uptake of NEFA into the liver to support higher rates of TG synthesis in TC individuals. It is speculated that the higher postprandial NEFA levels in TT carriers in part reflect their higher LPL-mediated TRL hydrolysis, with associated greater release of fatty acids into the circulation, and are consistent with the differences in TG values discussed above. However, further work is needed to gain an understanding of the influence of apoA-V and its gene variants on NEFA metabolism.

A recognized limitation of the current study is that participants were excluded if they were hyperlipidemic

(total cholesterol > 8 mmol/l, TG > 4 mmol/l) on hyperlipidemic mediation or had prediagnosed diabetes or CVD. Therefore, the individuals most likely to be sensitive to the impact of genotype may have been excluded, which would reduce the strength of the genotype-lipid associations observed.

In conclusion, the current study indicates that the apoA-V -1131T>C SNP is significantly associated with postprandial lipemia. To date, genetic susceptibility to hypertriglyceridemia has been attributed to variation in the LPL gene or that of its cofactor apoC-II and in some cases to apoE2 homozygosity. This study suggests that genotyping of individuals for the apoA-V -1131T>C SNP in addition to LPL, apoC-II, and apoE may be of clinical interest, in particular in those with a family history of diabetes or CVD. It would help identify individuals at high risk of developing exaggerated postprandial lipemia before presenting with clinical symptoms, which could result in more frequent monitoring and earlier intervention in the relevant subgroups. This does not rule out the possibility that variants in the apoC-III locus may have some independent effect, but it strongly suggests that apoA-V variants may be more important, in agreement with other studies that have examined fasting TG levels. Further work characterizing the strength and molecular basis of, and the impact of diet and other environmental factors on, apoA-V genotype-TG associations is merited.

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