LPL promoter −93T/G transition influences fasting and postprandial plasma triglycerides response in African-Americans and Hispanics

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Abstract The lipoprotein lipase (LPL) promoter −93T/G transition has previously been reported as having a triglyceride (Tg)-lowering effect, whereas the D9N variant has been shown to have a Tg-raising effect. These two variants were studied in 66 healthy subjects of Hispanic and 42 subjects of African-American origin, who had participated in a study of postprandial lipemia. While the allele frequency of the −93G was significantly different in the Hispanics and African Americans (0.09: 95% CI 0.04–0.13 and 0.28: 95% CI 0.19–0.38; *P* = 0.0001, respectively), the N9 allele frequency was not different (0.06: 95% CI 0.02–0.1 and 0.05: 95% CI 0.002–0.093, respectively). Linkage disequilibrium between the −93T/G and D9N was highly significant in Hispanics (Δ = 0.67. *P* = 0.0001), compared to Δ = 0.09 (NS) in African-Americans. In the combined group, compared to individuals with the common genotype (TT/DD; n = 71) with fasting plasma Tg of 1.34 (±4.5% SEM) mmol/l, carriers of the G/D haplotype (TG/DD + GG/DD; n = 25) had significantly lower plasma Tg levels of 1.08 (±10% SEM) mmol/l (*P* < 0.02). After the fat meal, compared to individuals with neither mutation, TT/DD, the effect of the G/D haplotype was to reduce significantly postprandial Tg (*P* < 0.036). Retinyl palmitate concentration at 5 hrs was significantly lower in G/D carriers than TT/DD individuals (*P* < 0.05). The lipid-raising effect of the N9 allele in carriers of the −93G (TG/DN + GG/DN) and effect on postprandial Tg clearance was not significant in this group. Thus carriers of the G/D haplotype have lower fasting plasma Tg and reduced alimentary lipemia. This allele may be associated with reduced risk of coronary artery disease.—Talmud, P. J., S. Hall, S. Holleran, R. Ramakrishnan, H. N. Ginsberg, and S.E. Humphries. **LPL promoter −93T/G transition influences fasting and postprandial plasma triglycerides response in African-Americans and Hispanics.** ***J. Lipid Res.*** **1998.** 39: **1189–1196.**

Supplementary key words LPL promoter variant • postprandial response • plasma Tg levels • African-Americans • Hispanics

Lipoprotein lipase (LPL) plays a central role in the catabolism of triglyceride-rich lipoproteins (TRLP) by hydrolyzing triglyceride (Tg) to glycerol and free fatty acids for energy use and storage in muscle and adipose tissue. There is strong evidence that in healthy individuals, postheparin plasma LPL (PHLPL) correlates well with fasting Tg levels (1–3), and LPL activity influences the clearance of Tg postprandially (4).

To date, more than 60 mutations resulting in inactive LPL have been identified (5–7). Complete LPL deficiency, familial lipoprotein lipase deficiency (FLLD), is a rare recessive disorder leading to chylomicronemia and often the complication of pancreatitis, resulting from the severe increase in plasma Tg due to the inability to hydrolyze TRLP (5). The frequency of FLLD is estimated to be one per million of the population with heterozygosity for the mutations in the order of one in 500 individuals. In a study of a large FLLD kindred, heterozygosity for the LPL mutation was associated with a pattern of combined lipidemia, only evident in carriers over the age of 40 years (8). Furthermore studies in the US (9) and the UK (10) have demonstrated that approximately one fifth of patients with familial combined hyperlipidemia or combined hyperlipidemia have LPL activity and mass below the 10th percentile for the general population. This cannot be explained by heterozygosity for FLLD mutations alone and common variations in the LPL gene that could influence LPL activity measures were predicted. Recently, two common variants within the coding region of the LPL gene, an Asn for Asp change at residue 9 (D9N) (11) and a Ser for Asn change at residue 291 (N291S) (12, 13), were identified with a combined population frequency of 3–6%. Both mutations are associated with raised plasma Tg (11, 13–17) and/or lower plasma HDL levels (12, 14, 17) and may predispose to FCHL and the risk of coronary

Abbreviations: TRLP, triglyceride-rich lipoproteins; Tg, triglyceride; PHLPL, postheparin plasma lipoprotein lipase; FLLD, familial lipoprotein lipase deficiency; LPL, lipoprotein lipase; CAD, coronary artery disease; HDL, high density lipoprotein; SSCP, single strand conformational polymorphism; FCHL, familial combined hyperlipidemia; AUC, area under the curve; FFA, free fatty acids; RP, retinyl palmitate.

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artery disease (CAD) (11, 12). In contrast, the S447X polymorphism in the LPL gene, with carrier frequency of 10–20% (18, 19), is associated with lower plasma Tg and raised HDL (18). This allele may be protective against CAD risk.

In order to identify additional common LPL variants, we screened the promoter of the LPL gene, using single strand conformational polymorphism (SSCP) (20), and identified a single common nucleotide transition, T to G at position −93 from the transcription start site. This −93T/G variation, together with two rare promoter mutations at positions −39 and −53 was identified by Yang et al. (21). Identifying carriers of −93T/G in healthy Caucasian men revealed that there was very strong linkage disequilibrium between the −93G and the LPL exon 2 G_{281} to A substitution resulting in the N9 variant (D = 0.91, P = 0.0001) (20). However, as the allelic association was not complete, we could identify individuals who carried the −93G on a wildtype D9 background, thus enabling us to establish the phenotypic effect of the −93G/D9 (G/D) haplotype in healthy Caucasian men (20). Carriers of this G/D haplotype had lower plasma Tg levels, compared to non carriers of either variant (TT/DD) (20) which could be explained by in vitro studies, using luciferase as a reporter gene, showing that the −93G promoter had increased expression activity (20).

Because humans spend as much as 75% of time in a postprandial state, the true physiological impact of variation in the LPL gene may be more evident after a postprandial challenge when the ability of LPL variants to clear dietary Tg may be better assessed. Several studies have investigated the effect of LPL mutations on postprandial response in heterozygotes for the null alleles (22), and in carriers of S291 (14, 23) and N9 (14). In individuals of Afro-Caribbean origin, the allele frequency of the −93G was 23-fold higher than that seen in Caucasians, with greatly reduced allelic association with N9 (20). Thus metabolic studies and the effect of variation at position −93 on lipid traits are more feasible in individuals of African origin. This population facilitated the study of the effects of the −93G variant independent of the N9 variant. In this study we have investigated the combined effects of both −93T/G and D9N variants on plasma lipid levels, both in the fasting and postprandial state, in subjects of both African and Hispanic origin who had participated in a previously reported postprandial trial (24).

METHODS

Study Population

A detailed description of the subject recruitment and inclusion and exclusion criteria for participation in the postprandial trial has been reported by Ginsberg et al (24). Briefly men and women of all ethnic groups with an age range of 20–75 years, who had been referred to either the Columbia-Presbyterian Medical Center or Harlem Hospital Center of the City of New York for ECG exercise testing or a thallium stress test, were recruited for the study. This group was considered to be representative of a population referred for diagnostic evaluation of CAD prior to dietary or exercise lifestyle changes. All subjects underwent symptom-limited treadmill exercise according to the Bruce protocol. In total, 205 individuals were recruited into the study and, on the basis of the ECG response, individuals were classified as cases or controls. Thallium stress test results were used as a basis for the classification when they were available, irrespective of accompanying ECG results. However, in this present study, only those individuals classified as controls (n = 155) were considered. In the final analysis we excluded those individuals who were either of Caucasian or Asian ethnic origin, as the frequency of the −93G/D9 haplotype was very low in those groups (1/43), as well as carriers of the N291S mutation, leaving in total 108 control individuals of African-American or Hispanic origin. There was no bias in how the African-American and Hispanic controls were chosen for the analysis. The characteristics of the African-American and Hispanic controls included in this study are given in **Table 1**.

Postprandial lipemia protocol

This protocol was carried out at the General Clinical Research Center at the Columbia-Presbyterian Medical Center (24). All subjects arrived having fasted overnight and fasting blood samples were taken as well as anthropometric measures. They were then given a fat formula that was composed of heavy whipped cream, ice cream, safflower oil, and a powdered protein source (Promod, Ross Laboratories). The nutrient composition of the formula, based on a body surface area of 2 m^2, included 105 g fat with 52 g saturated fat, 48 g carbohydrate, 32 g protein and 300 mg cholesterol, providing 1265 calories. Vitamin A (100,000U/2 m^2 body surface) was added in the form of Aquasol A (Astra Pharmaceutical). This fat formula was consumed in 15 minutes and blood samples taken at 2, 3.5, 5 and 8 h.

Lipoprotein measurements

Blood was taken into sterile EDTA tubes for plasma and DNA extraction, and into sterile tubes for serum sampling. The detailed protocol of sample handling has previously been reported (24). Standard methodologies for determining cholesterol, Tgs in whole plasma, HDL-C, and Tgs in d < 1.006 determination were followed (24). Plasma retinyl palmitate (RP) levels were

TABLE 1. Characteristics of the subjects by ethnic origin and gender

Variable	African-American		Hispanic	
	Male	Female	Male	Female
Age ± SD, yr	47 ± 8	55 ± 10	51 ± 9	53 ± 11
Number	19	23	22	44
BMI ± SEM, kg/m²	27.5 ± 5.5	28.7 ± 5.6	27.4 ± 3.9	29.6 ± 6.4
WHR ± SEM	0.92 ± 0.07	0.86 ± 0.06	0.95 ± 0.06	0.88 ± 0.09
Tg ± %SEM, mmol/1	1.15 ± 9	1.01 ± 7.7	1.36 ± 10	1.47 ± 5.6
TC ± SEM, mmol/1	4.85 ± 0.97	5.5 ± 1.0	4.9 ± 0.94	5.3 ± 1.0
LDL-C ± SEM, mmol/1	3.3 ± 0.88	3.5 ± 0.98	3.3 ± 0.73	3.4 ± 0.96
HDL-C ± SEM, mmol/1	1.0 ± 0.19	1.45 ± 0.39	0.94 ± 0.22	1.2 ± 0.29

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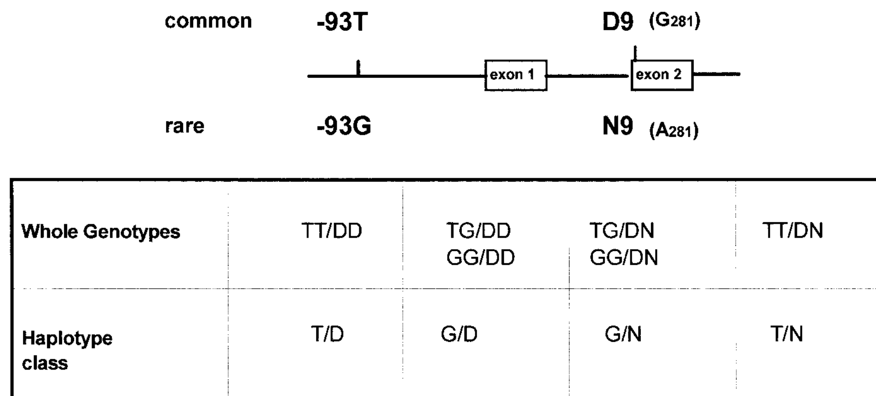

Fig. 1. Map of the LPL gene showing the variant sites at −93 in the promoter and and residue 9 in exon 2. The genotypes identified in the study and the four deduced haplotypes are presented.

measured by reverse phase high performance liquid chromatography as described by Bieri, Tolliver, and Catignani (25).

DNA extraction and genotyping

DNA was extracted from leucocytes using the salting out method (26). DNA samples were sent to Division of Cardiovascular Genetics, University College London, London, UK for LPL genotyping. Polymerase chain reactions (PCRs) for identification of nucleotide −93T/G and the D9N variants were performed on an MJ Research PTC-220 thermal cycler, using oligonucleotides and PCR conditions as reported previously (20). Briefly −93G introduces a HaeIII restriction enzyme cutting site, distinguishing HaeIII digested −93G PCR product (153 bp and 50 bp) from −93T (203 bp). For identification of the D9N variant, the invariant TaqI restriction enzyme cutting site, 6 bp downstream of the nucleotide responsible for the D9N variation, was abolished by PCR priming and identification of the N9 allele after TaqI digest gave fragment sizes of 52 and 24 bp compared to D9 allele fragment size (absence of TaqI site) of 76 bp. LPL N291S genotyping (14) and apoE genotyping (27) were carried out as previously described. Microtitre Array Diagonal Gel Electrophoresis (MADGE) was used for high throughput genotyping (28).

Statistical analyses

Biometric data previously obtained (24) were used for comparison of carriers with non-carriers at both −93 and residue 9. The gene-counting method with a χ^2 test with Yates correction was used to compare the frequency of the −93G variant allele between the different groups. Linkage disequilibrium between the two variant sites (−93T/G and D9N) was estimated using the correlation coefficient $\triangle$ (29). A maximum likelihood algorithm method was used to determine the most likely ‘phase’ of the −93T/G and D9N mutations in those individuals heterozygous for both (30). As allelic effects were considered to be the same in both groups, the effect of the −93G independent of the N9 variant was estimated in the combined sample. Differences in outcome measures (lipid levels, incremental area under the curve above fasting) were analyzed among three haplotype groups; T/N haplotype, with only three subjects, was excluded from the analysis. As lipid levels were partly determined by gender and BMI, these two factors and their interaction (the possibility that the BMI effect is different for men and for women) had to be taken into account in the analysis. Therefore, an analysis of variance with haplotype, gender, BMI, and gender times BMI was carried out for each outcome measure; the GAM procedure of SAS was used for this purpose. To test differences in Tg concentrations, values were log-transformed prior to statistical analysis. Statistical significance was considered to be at the $P <$ 0.05 level.

RESULTS

All individuals were genotyped for variation at −93, residue 9, residue 291, and apoE. Those individuals who were carriers of S291 variant were excluded from the outset. The relationship of −93 and residue 9 and the genotypes and haplotype classes identified in the study are presented in **Fig. 1**. The genotype and haplotype distributions in the African-Americans and Hispanics are presented in **Table 2**. On the basis of this, the allele frequency of the −93G was calculated to be 0.28 (95% CI 0.19–0.38) in the African-Americans and 0.09 (95% CI 0.04–0.13) in the Hispanics; this difference was statistically significant ($P < 0.001$). However, there was no N9 allele frequency difference between the two groups; 0.05 (95% CI 0.002–0.09) in the African-Americans and 0.06 (95% CI 0.02–0.1) in Hispanics. Linkage disequilibrium between the −93G and N9 variants was highly significant in the Hispanics ($\triangle = 0.67$; $P <$ 0.0001) but not in the African-Americans ($\triangle = 0.09$, NS).

Because we assumed that the allelic effects of both the −93G and N9 variants would not differ between the two ethnic groups, and to give a large enough sample size to have the power to identify a haplotype effect, all lipid data were analyzed on the sample of Hispanics and African-Americans combined. To test this, data was reanalyzed excluding the Hispanics and no difference was seen in the results, thus confirming homogeneity.

TABLE 2. Genotype distribution of the −93T/G and D9N mutations in the two ethnic groups

Group	Total	(TT/DD)[a] T/D[b]	(TG/DD+ GG/DD)[a] G/D[b]	(TG/DN+ GG/DN)[a] G/N[b]	(TT/DN)[a] T/N[b]
African-American	42	18	19 + 1	1 + 1	2
Hispanic	66	53	5 + 0	7 + 0	1

[a] Genotype.
[b] Haplotype.

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TABLE 3. Mean fasting plasma Tg, HDL-C, TC, and LDL-C levels for the combined African-American and Hispanic groups according to haplotype defined by −93T/G and D9N

	T/D		G/D		G/N		T/N	
Variable	mmol/l	n	mmol/l	n	mmol/l	n	mmol/l	n
Tg ± %SEM	1.34 ± 4.5%	71	1.08 ± 10%	25	1.36 ± 13%	9	1.33 ± 22%	3
HDL-C ± SEM	1.17 ± 0.041	70	1.20 ± 0.072	25	1.09 ± 0.06	9	1.06 ± 0.08	3
TC ± SEM	5.23 ± 0.12	70	5.23 ± 0.22	25	4.65 ± 0.29	9	4.7 ± 0.20	3
LDL-C ± SEM	3.41 ± 0.11	70	3.45 ± 0.20	25	2.88 ± 0.24	9	3.00 ± 0.05	3

General linear model: For Tg: effect of haplotype, $P = 0.05$; pairwise *t*-test: T/D versus G/D, $P = 0.02$, TD versus G/N, P =NS, G/D versus G/N, P = NS. For HDL-C, TC, and LDL-C: effect of haplotype, P = NS; pairwise *t*-tests, P = NS.

The fasting plasma lipid values are given for each haplotype class in **Table 3**, and represent data pooled as in Table 2 and Fig. 1. Haplotype T/N was not included in further analyses because of its small sample size. When all three remaining haplotype groups were considered together in a general linear model taking haplotype, BMI, and BMI by sex into account, for fasting Tg, the overall effect was highly significant ($P = 0.0001$) with haplotype having a significant effect ($p = 0.049$) and BMI × gender having a highly statistically significant effect ($P = 0.002$). This gender effect was due to the strong correlation between Tg and BMI seen in the males but not the females (data not shown). Pairwise analysis of the effect of haplotype showed that compared to individuals with the common haplotype T/D, subjects with the G/D haplotype had significantly lower plasma Tg (1.34 mmol/l vs 1.08 mmol/l, $P = 0.019$) (Table 3) . However, no difference was seen between individuals with both rare variants (G/N) and those with the common haplotype T/D. Thus the previously reported Tg raising effect of the N9 mutation was not evident in this sample. The same analyses carried out on fasting HDL-C levels showed a significant effect in the overall model ($P = 0.0001$) but this could be explained entirely by the effect of gender. There was no haplotype effect on HDL in the pairwise analysis, nor was there a haplotype effect on fasting cholesterol, LDL-C, and apolipoprotein (apo) B. There was also no haplotype effect on apoA-I levels (results not shown). To test whether apoE genotype (results not shown) had any effect on fasting or postprandial Tg, analyses were carried out using apoE genotype as a factor in the regression model. ApoE genotype did not contribute significantly to the regression analysis.

In our previous study (20) no carriers of the TT/GN genotype, that is individuals with the N9 variant independent of −93G, were identified. It is very interesting to note that in the present study three individuals with the genotype TT/GN were identified. Investigation of the effect of the N9 on a common −93T background on plasma lipid levels would be of special interest. However, because of the small number of subjects with this haplotype, the data, which are shown in Table 3, were not included in the statistical analyses.

After the ingestion of the fat meal, postprandial concentrations of Tg and RP were followed for 8 h. The results, represented by the area under the curve (AUC) calculated for each of the three haplotype classes, are presented in **Table 4**. In the pairwise analysis, carriers of the G/D haplotype (TG/DD and GG/DD individuals) had a significantly smaller AUC for Tg clearance than T/D individuals with neither variant (TT/DD), (5.22 mmol/l/h, vs 7.69 mmol/l/h, respectively; $P = 0.036$). There was no difference in Tg clearance in carriers of both rare variants G/N (TG/DN and GG/DN) compared to other haplotype groups. These data are represented in **Fig. 2**. When the clearance of RP was considered, there was no statistically significant difference in AUC in the pairwise analysis by haplotype. However, at 5 h, the concentration of RP was significantly lower in the G/D group compared to the T/D group ($P = <0.05$). The clearance of RP over time in the three genotype classes is shown in **Fig. 3**.

After the 8-h postprandial sample had been taken, a postheparin blood sample was taken and LPL activity was measured in a subset of individuals. These results show that individuals homozygous for the D9 allele, TT/DD (n = 28) or TG/DD + GG/DD (n = 9) had PHLPL activity measures of 14.25 mmol FFA/ml per hr (SD ± 6.2) and 14.08 mmol FFA/ml per hr (SD ± 10.9), respectively; while individuals carrying the N9 allele (TG/DN + GG/DN (n = 6) or TT/DN (n = 2) had LPL activity of 9.4 mmol FFA/ml per hr (SD ± 4.6) and 10.6 mmol FFA/ml per hr(SD ± 10.3), respectively. However, the number of samples was small and the differences were not statistically significant.

DISCUSSION

In this study we have investigated the impact of variation at both position −93 in the LPL promoter and residue 9 in the LPL protein on fasting Tg and postprandial response in individuals of African-American and Hispanic

TABLE 4. Area under the curve for postprandial Tg clearance and RP clearance for the combined African-American and Hispanic groups according to −93T/G and D9N haplotypes

	Haplotype		
	T/D n = 71	G/D n = 25	G/D n = 9
Tg (mmol/1/h) ± %SE	7.69 ± 8.6%	5.22 ± 18%	7.05 ± 25%
RP (mg/d1/h) ± %SE	305.03 ± 8.5%	261.6 ± 12%	305.5 ± 19%

Pairwise *t*-test: T/D versus G/D, $P = 0.03$; T/D versus G/N, P = NS; G/D versus G/N, P = NS. For RP: effect of genotype, P = NS; pairwise *t*-test, P = NS.

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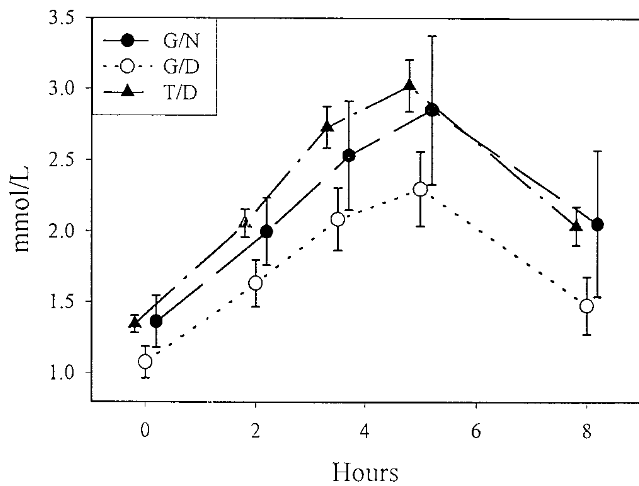

Fig. 2. The clearance of postprandial Tg with time according to −93T/G and D9N haplotypes: T/D (n = 70), G/D (n = 25), G/N (n = 9) (T/N not included). Data represent the incremental area above fasting Tg.

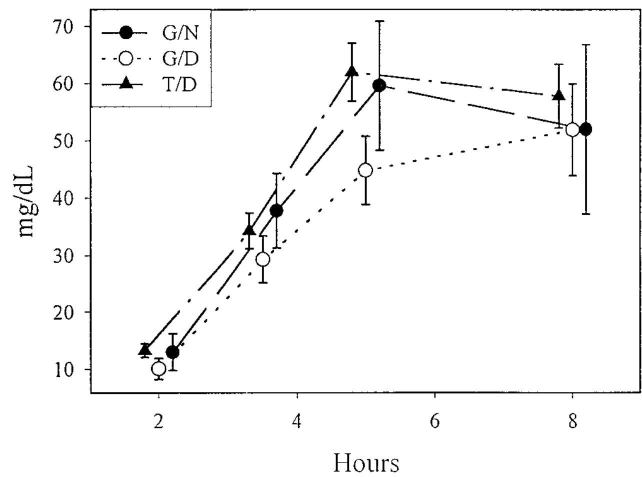

Fig. 3. The clearance of postprandial retinyl palmitate with time according to −93T/G and D9N haplotypes: T/D (n = 70), G/D (n = 25), G/N (n = 9) (T/N not included).

origin residing in New York. Because of the previously identified strong allelic association between these two variant sites within the LPL gene, it was essential that any study of one of these variants must include the other. We previously reported that the allele frequency of −93G was 23-fold higher in Afro-Caribbeans of West Indian origin living in the UK compared to Caucasians (0.39 vs 0.017 $P < 0.0001$)) (20). In the African-Americans studied here, the allele frequency was slightly lower (0.28) but not significantly different from that reported in the UK Afro-Caribbeans ($\chi^2 = 2.72$; P = NS) and this might reflect the gene dilution over the past 300 years since the movement from Africa, or possibly different points of origin within the African continent. It is now clear, from sequence comparisons of the LPL promoter in primates (20), that the G is the nucleotide present at position −93 and thus is likely to represent the ancestral allele. In the Hispanics, the −93G allele frequency of 0.09 is intermediate to that of individuals of African or Caucasian origin and may reflect admixture. The allele frequency of the N9 variant was no different in the two groups but in both groups this was significantly higher when compared to the overall combined carrier frequency reported in healthy Caucasians, pooled from various studies (100/5846 alleles, with an allele frequency 0.017 (95% CI 0.013–0.02) (11, 14, 15), compared to the Hispanics $P < 0.001$, compared to the African-Americans $P < 0.05$). A possible confounder to analyzing the data of the pooled sample would be if there were socio-economic or dietary differences between the ethnic groups. However, to control for this all African-Americans and Hispanics came from northern Manhattan. In addition, analyses showed that neither pre-diet nor pre-activity levels (determined by questionnaire) affected the postprandial area under the curve (H. N. Ginsberg, unpublished results). In order to check that there were no confounders such as ethnic specific environmental or genetic differences, the data were reanalyzed, excluding the Hispanics, and no difference was seen in the results, thus suggesting homogeneity. However, these data would be strengthened if they were confirmed in a larger cohort.

Allelic association between −93G and N9 variants was tight in the Hispanics ($\Delta = 0.67$) compared to that in the African-Americans ($\Delta = 0.09$). However, the allelic effect was not expected to be different and so the analyses represent those carried out on the sample as a whole. The results confirm the finding from our previous study in Caucasians, namely, that carriers of the G/D haplotype have significantly lower fasting plasma Tg levels than carriers of neither rare variant (TT/DD) (20). HDL is known to be inversely related to plasma Tg levels and in studies of the D9N and N291S mutations an effect on both raised Tg (11, 13–16) and/or lower HDL levels (12, 14) has been reported. However, no significant effect of the G/D haplotype on fasting HDL levels was seen, although levels are slightly higher than in T/D individuals while G/N individuals have slightly lower HDL-C levels.

Previous studies have shown that variation at residues 9 (now defined by haplotype G/N) and 291 are associated with raised Tg levels, compatible with in vitro experiments showing reduced secretion of the LPL-N9 (11, 31, 32) and reduced stability of LPL-S291 (12, 31, 33). As we aimed to study the effect of −93T/G and N9 specifically, individuals carrying the S291 mutation were excluded from the analyses at the outset. ApoE genotype had no effect on the regression equation and therefore could be excluded from influencing the effect of LPL genotype. We have evidence from a study of 500 healthy individuals of African origin, genotyped for both the –93T/G and S447X variants, that there is no allelic association between these two variant LPL sites ($\Delta = 0.07$, NS), confirming that the effect associated with −93G was independent of variation at residue 447 (P. J. Talmud, S. Hall, F. Capucchio, and D. Cook, unpublished results).

The G/D haplotype is associated with lower plasma Tg and this is also in accord with in vitro studies in both a rat smooth muscle cell line (A10) and human adrenal cell

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line (NCIH 295) where, compared to the −93T promoter, luciferase reporter gene activity driven by the −93G promoter was shown to be increased by 24% ($P = 0.05$) and 18%, respectively (20). These results do not agree with those from Yang et al (34) who reported that the −93G promoter showed 50% reduced reporter gene expression in THP-1 cells (a monocyte cell line) and C2C12-F3 cells (a myoblast cell line). This may reflect a tissue specific difference in the expression of LPL. Reduced −93G promoter activity, reported by Yang et al. (34), would be expected to result in increased Tg levels in carriers of the G/D haplotype, and this was not seen in either the present or the previous study (20).

The postprandial clearance of Tg as a measure of lipolysis and retinyl palmitate (RP) as a measure of remnant disappearance (in part dependent on lipolysis) was also investigated. Compared to carriers of neither variant (TT/DD), G/D carriers had significantly lower postprandial lipemia (AUC $P = 0.03$), suggesting improved lipolysis after a fat meal. This suggests that, in response to an increase of TRLP after the fat intake, −93G LPL expression is up-regulated even more than at fasting and results in a more rapid lipolysis of Tg. Although the difference in AUC by haplotype did not reach statistical significance in RP clearance, the 5-hr difference was statistically lower in G/D carriers compared to TT/DD individuals, suggesting an effect of G/D on RP clearance. This 5-h measure coincides with the peak of postprandial LPL activity (35) which accords with insulin infusion studies showing that insulin stimulation of LPL peaks at 4–6 h (3). Although in carriers of the G/D haplotype, RP levels rose slightly at the 8 h time point, which could be interpreted as reflecting an accumulation of remnants, the study by Karpe et al. (36) strongly suggests that time points later than 6 h after the fat load do not necessarily represent the postprandial clearance of RP, but may in fact represent, in addition, RP accumulated in small chylomicrons synthesized de novo from the intestine, and not only the result of remnant accumulation from large chylomicrons.

From our previous study, we concluded that when −93G and N9 are co-inherited, the increased LPL expression due to the −93G promoter leads to an overexpression of a secretion-defective protein thus amplifying the LPL-N9 deficiency (20). In this sample the effect of the G/N haplotype (TG/DN and GG/DN individuals) on fasting Tg was no different from TT/DD individuals. This could reflect small sample size (n = 9) or it is possible, due to gene–gene or gene–environment interaction, that this effect is not as strong in the present sample. Thus, even though the frequency of the N9 variant is significantly higher than in Caucasians, it is possible that the effect of the N9 in individuals of African and Hispanic origin is milder. A search for variation in the coding region of the LPL gene in individuals of African origin might be of interest as the possibility also remains that these subjects carry a common functional variation in the LPL gene that is rare or not present in Caucasians.

It is of interest to note that three individuals with the genotype TT/DN, i.e., carriers of the N9 allele on the common −93T background, were identified in this sample. This haplotype was not seen in the 1565 Caucasians or 91 Afro-Caribbeans previously studied (20). However, because of the small number, it was not possible to include them in the statistical analyses. Their fasting plasma Tg levels were 1.33 mmol/l (±22% SEM) and no different from the TT/DD individuals. It would be of interest to study a larger group of individuals of African/Hispanic origin to assess the true lipid-raising effect of the N9 variant on a −93T background. Our prediction, however, is that N9 on a common −93T allele would not have a very strong effect on plasma Tg and it is only in combination with increased expression, resulting from the −93G promoter, that overproduction of the secretion-defective LPL-N9 results in raised plasma Tg levels.

Several studies have investigated the effect of LPL variants on postprandial response, with the effect being most exaggerated in carriers of mutations leading to LPL deficiency. Thus, in a study of eight E188 carriers, the most common LPL mutation leading to FLLD (37), with half normal LPL activity, postprandial lipemia was twice that of the eight non-carriers (22). The largest postprandial study of S291 and N9 carriers to date is the European Atherosclerosis Research Study II (EARS II), an offspring study of 'cases' whose fathers had had a premature MI versus age-matched controls (14). The effect of the S291 variant on postprandial Tg clearance was to reduce clearance and this was seen only in the cases of carriers versus non-carriers, but not in the controls, and was most pronounced in the late postprandial stage. No differences in postprandial Tg concentrations between carriers of the N9 allele and non-carriers in cases or controls were observed (14). The EARS II study clearly demonstrated that the effects of the two common LPL mutations N9 and S291 on plasma lipid metabolism were quite different. For S291 it is postulated that the effect is through lowered LPL stability, leading to a reduction in the amount of active LPL available for hydrolysis of chylomicrons and VLDL and a poorer postprandial response. The effect of the N9 mutation (in carriers of the G/N haplotype), while affecting fasting Tg (and weaker effects on HDL-C) appears not to be mediated through effects on postprandial lipid metabolism. One possible explanation for this is the fact that the mutation results in delayed (reduced) secretion of an apparently normally active enzyme. Thus it is postulated that after an overnight fast, capillary heparan sulfate proteoglycans have maximum LPL-N9 and therefore after a fat load there is sufficient LPL-N9 to meet the demand postprandially. In the present study there was no difference in the postprandial response seen in G/N carriers, but once again this could merely reflect, due to the small sample, the lack of power to see a difference. We propose that a successive oral fat load would unmask the moderate decrease in activity in G/N carriers. Some support for the hypothesis comes from the PHLPL activity measures taken after the 8-h postprandial blood sample, that, in fact, is a measure of the quantity (as represented by the activity) of LPL remaining after the postprandial trial period. Although the present data do not

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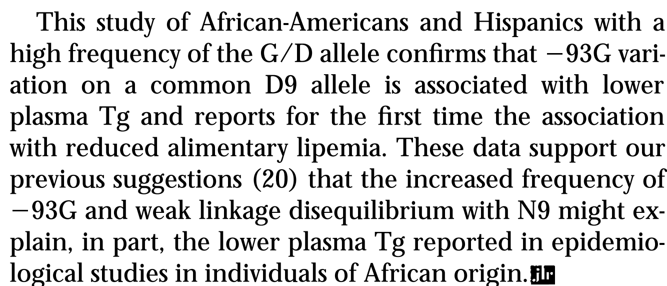
reach statistical significance, the expected trend is seen, namely that carriers of the G/N or T/N haplotypes have, on average, 35% lower LPL activity than carriers of the G/D or T/D haplotype. Thus, although these results are preliminary, they suggest that variation at residue 9 and not −93 determines the levels of postheparin LPL activity after a fat meal, and need to be followed in a larger study either, by PHLPL activity measures after the postprandial trial period or by challenging with a second fat meal. Because for healthy subjects meals are usually taken during the day only a few hours apart, this would suggest that carriers of G/N allele would have reduced postprandial clearance under normal free-living conditions. Hence the studies performed to date may not provide good models for the risks of atherosclerosis and CAD experienced by G/N carriers.



This study of African-Americans and Hispanics with a high frequency of the G/D allele confirms that −93G variation on a common D9 allele is associated with lower plasma Tg and reports for the first time the association with reduced alimentary lipemia. These data support our previous suggestions (20) that the increased frequency of −93G and weak linkage disequilibrium with N9 might explain, in part, the lower plasma Tg reported in epidemiological studies in individuals of African origin.

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