Two DNA polymorphisms in the lipoprotein lipase gene and their associations with factors related to cardiovascular disease

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Abstract Lipoprotein lipase (LPL) plays a crucial role in plasma lipoprotein processing by catalyzing the hydrolysis of core triglycerides of chylomicrons and very low density lipoproteins. Several polymorphic restriction sites have been reported in the LPL gene, including those identified by the enzymes HindIII and PvuII. We have determined the HindIII and PvuII polymorphisms in diabetic (D) and non-diabetic (ND) Hispanics (D = 195; ND = 384) and non-Hispanic Whites (D = 76; ND = 539) from the San Luis Valley, Colorado. Both polymorphisms showed comparable gene frequencies between diabetics and non-diabetics, and between the two ethnic groups. The HindIII and PvuII polymorphisms were in strong linkage disequilibrium in both Hispanics and non-Hispanic Whites ($P < 0.001$). We estimated whether the two DNA polymorphisms have significant impact in determining interindividual differences in plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting glucose, and fasting insulin. Plasma triglyceride levels varied significantly among the HindIII genotypes in the normoglycemic sample. There was a clear gene dosage effect among the three HindIII genotypes, with the (−/−) genotype having the lowest and the (+/+) genotype having the highest triglyceride levels; these levels were intermediate in the (+/−) genotype. The average effect of the (−) allele of the HindIII polymorphism was to lower triglycerides by 12.85 mg/dl in non-Hispanic White males, 8.06 mg/dl in non-Hispanic White females, 10.91 mg/dl in Hispanic males, and 12.47 mg/dl in Hispanic females. The HindIII polymorphism also showed a significant association with HDL-cholesterol levels in the normoglycemic sample. The (+/+) genotype was associated with lower levels of HDL-cholesterol although no clear gene dosage effect was seen. Unlike the HindIII analysis in the normoglycemic sample, no significant association was detected between PvuII genotype and any quantitative trait in the normoglycemic sample. Among diabetics, the HindIII polymorphism was significantly associated with fasting insulin levels in Hispanic males only ($P = 0.009$), while the PvuII polymorphism showed no association with any quantitative trait level. Thus, this study confirms an association between genetic variation at the LPL locus and plasma triglyceride levels.—**Ahn, Y. I., M. I. Kamboh, R. F. Hamman, S. A. Cole, and R. E. Ferrell.** Two DNA polymorphisms in the lipoprotein lipase gene and their associations with factors related to cardiovascular disease. *J. Lipid Res.* 1993. **34:** 421–428.

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Lipoprotein lipase (LPL) plays a key role in lipid metabolism. By hydrolyzing plasma triglycerides, LPL provides the fatty acids to tissues for oxidation, storage, and secretion and influences the maturation of circulating lipoproteins. Recently, LPL has been shown to enhance the binding of triglyceride-rich chylomicrons to the low density lipoprotein receptor-related protein and thus may be important in chylomicron metabolism because of its lipolytic activity, and because of its structural properties (1). The activity of LPL is regulated by nutrients and hormones, for example, glucose and insulin (2–5).

The LPL gene is located on chromosome 8p22 (6). It is about 35 kb in length and contains 10 functionally differentiated exons (7, 8). Several restriction fragment length polymorphisms (RFLPs) have been reported at the LPL locus (9–15). Association studies with RFLPs at this locus have reported that the HindIII polymorphism is associated with hypertriglyceridemia (16) and coronary heart disease (17); and with levels of total cholesterol and high density lipoprotein (HDL) cholesterol (12). The PvuII site has been reported to be associated with variation in plasma triglyceride levels (16).

Because of its intimate involvement in lipid metabolism, the LPL gene is considered to be an important candidate

Abbreviations: N, number of individuals; LPL, lipoprotein lipase; BMI, body mass index; Tchol, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Tg, triglycerides; Fast G, fasting glucose; Fast I, fasting insulin; RFLP, restriction fragment length polymorphism; NHW, non-Hispanic White; NIDDM, non-insulin-dependent diabetes mellitus.

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gene in determining the risk factor levels in metabolic disorders such as atherosclerosis, diabetes, and obesity. In a study of Hispanics (S. A. Cole, C. Aston, R. F. Hamman, and R. E. Ferrell, unpublished results), nonrandom association between a PvuII allele at the LPL locus and fasting insulin levels in Hispanic males was observed, and previous studies had reported an association between LPL variation and triglyceride levels. This study was undertaken to test whether genetic variation at the LPL locus was associated with fasting insulin levels in a larger sample and to test whether LPL variation had a significant impact on triglyceride levels in a large sample of normoglycemic and non-insulin-dependent diabetic (NIDDM) Hispanics and non-Hispanic Whites (NHW) from the San Luis Valley, Colorado.

MATERIALS AND METHODS

Study population

The San Luis Valley Diabetes Study (SLVDS) was designed to examine risk factors and prevalence of NIDDM using a case-control approach in the biethnic population (Hispanics and non-Hispanic Whites) of the San Luis Valley in Southern Colorado (18). The non-Hispanic White (NHW) population immigrated to the valley from several Northern European countries in the mid-1800s. The Hispanic population has lived in this area since the time of the Spanish land grants. Today there is little immigration to the area especially from Mexico, in contrast with conditions in Texas and Southern California (18). During the 150-year settlement in this valley, there has been little direct Amerindian-Hispanic genetic admixture. Serological data show that the proportion of Amerindian genes in NHW persons is about 3%, and 19% in the Hispanics (19).

Our sample consists of 923 nondiabetics (NHW = 539; Hispanics = 384) and 271 non-insulin-dependent diabetics (NHW = 76; Hispanics = 195). They were between 20 and 74 years of age. Details of the clinical and biometrical features of the studied samples are published (20, 21). These are summarized in **Table 1** for the normoglycemic sample and in **Table 2** for the NIDDM sample from the San Luis Valley, CO.

DNA typing

Fasting EDTA blood samples were collected from study subjects, and the white blood cells were isolated, frozen at −70°C, and shipped to Pittsburgh. DNA was extracted from the white cells by the procedure of Miller, Dykes, and Polesky (22). DNA samples were subjected to amplification by the polymerase chain reaction (PCR) in a Perkin-Elmer Cetus DNA Thermal Cycler (Norwalk, CT). We used two sets of primers. One set was derived from sequences between exons 8 and 9 in the lipoprotein lipase (LPL) gene to amplify the sequence around a HindIII restriction site in intron 8 (the forward primer was 5'-TTTAGGCCTGAAGTTTCCAC-3'; and the reverse primer was 5'-CTCCCTAGAACAGAAGATC-3') (23). The amplified fragment had a size of 1.3 kb. The other set was from the DNA sequences flanking a PvuII restriction site in intron 6 (the forward primer was 5'-TAGAGGTTG AGGCACCTGTGC-3'; and the reverse primer was 5'-GTGGGTGAATCACCTGAGGTC-3') (24). The amplified fragment in this case was 858 bp long.

The 50-μl reaction mixture contained 1 × PCR buffer (10 mM Tris, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$), dNTPs at 200 μM, 0.3 μM each primer, 0.5 μg genomic DNA, and 1.25 units of Taq DNA polymerase. Amplification of the region flanking the HindIII site was carried out for 33 cycles at 95°C for 1 min, at 60°C for 2 min, and at 72°C for 2 min. For amplification of the sequence around the PvuII site, the conditions were the same except for annealing at 70°C and 25 cycles. Amplified products were digested with HindIII or PvuII and the resulting fragments were separated on 2% agarose gels. After digestion with HindIII, the presence of the restriction site (+ allele) resulted in fragments of 600 and 700 bp. The presence of the PvuII site (+ allele) yielded fragments of 266-bp and 592-bp.

TABLE 1. Clinical features (mean ± SD) for the nondiabetic subjects in the SLVD study population

	NHW Nondiabetics		Hispanic Nondiabetics	
Variable	Males	Females	Males	Females
N	254	285	187	197
Age (yr)	52.5 ± 11.6	52.3 ± 11.6	52.5 ± 12.4	50.5 ± 12.7
BMI (kg/m²)	26.3 ± 3.5	24.9 ± 4.5	25.5 ± 3.9	26.0 ± 5.0
Fast G (mg/dl)	97.6 ± 7.8	92.7 ± 7.9	98.1 ± 9.4	93.3 ± 8.9
Fast I (μIU/ml)	11.7 ± 6.5	11.2 ± 8.0	12.5 ± 6.7	13.5 ± 8.1
Tchol (mg/dl)	213.8 ± 40.0	218.2 ± 43.7	216.1 ± 45.7	211.8 ± 40.6
HDL-C (mg/dl)	44.5 ± 10.8	56.9 ± 14.0	45.6 ± 12.1	53.0 ± 13.5
LDL-C (mg/dl)	140.3 ± 36.4	135.9 ± 39.2	139.7 ± 43.3	130.0 ± 36.9
Tg (mg/dl)	148.0 ± 36.4	129.2 ± 61.4	157.0 ± 72.4	139.2 ± 62.6

Abbreviations: Fast G, fasting glucose; Fast I, fasting insulin; Tchol, total cholesterol; Tg, triglycerides; NHW, non-Hispanic White.

TABLE 2. Clinical features (mean ± SD) for the NIDDM subjects in the SLVD study population

Variable	NHW Diabetics		Hispanic Diabetics	
	Males	Females	Males	Females
N	44	32	75	120
Age (yr)	59.1 ± 8.5	60.9 ± 9.2	56.2 ± 11.3	58.5 ± 10.7
BMI (kg/m²)	29.4 ± 4.4	30.4 ± 5.6	28.0 ± 4.2	30.0 ± 5.2
Fast G (mg/dl)	188.4 ± 72.2	186.4 ± 67.4	184.0 ± 71.5	190.4 ± 72.5
Fast I (μIU/ml)	26.9 ± 22.9	34.6 ± 35.6	27.6 ± 41.2	29.1 ± 28.4
Tchol (mg/dl)	204.6 ± 39.6	208.4 ± 42.8	213.9 ± 37.3	238.1 ± 47.2
HDL-C (mg/dl)	37.8 ± 11.6	47.3 ± 11.3	40.1 ± 11.4	47.9 ± 14.8
LDL-C (mg/dl)	129.9 ± 40.3	121.8 ± 36.6	131.9 ± 33.8	143.6 ± 41.4
Tg (mg/dl)	239.6 ± 188.4	198.0 ± 110.4	228.8 ± 133.3	246.8 ± 171.7

Abbreviations: Fast G, fasting glucose; Fast I, fasting insulin; Tchol, total cholesterol; Tg, triglycerides; NHW, non-Hispanic White.

Metabolic determinations

Lipid and carbohydrate levels were determined in the laboratories of the Clinical Research Center, University Hospital, Denver, CO, from fasting blood samples (overnight fasting > 8 h). Triglycerides were determined by enzymatic assay (25) and total cholesterol by the esterase-oxidase method (26). High density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic assay, after dextran sulfate and magnesium precipitation (27). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (28). Individuals with triglyceride levels ≥400 mg/dl were excluded from the samples. Fasting serum insulin level was obtained by radioimmunoassay (29), and the fasting glucose level by glucose oxidase (30).

Statistical analyses

Allele frequencies for each polymorphic site were estimated by gene counting. Heterozygosity (H) (31) and polymorphism information content (PIC) (32) were computed to estimate the informativeness of each polymorphism. Goodness of fit to Hardy-Weinberg proportions was tested by the log-likelihood ratio (33). The degree of nonrandom association between any two polymorphic sites at the LPL gene was determined by calculating the delta (pairwise) value (34) using maximum likelihood estimates of the haplotype frequencies. Haplotype frequencies were also computed by direct counting in individuals heterozygous for only a single site.

To evaluate the effect of each polymorphism on the variation of quantitative variables of interest, an analysis of covariance was carried out. The distributions of all the variables were tested for normality and the determination of the effects of covariates was done by stepwise regression. Age, smoking, and BMI were considered as covariates. BMI was computed as weight (kg) divided by height (m) squared (35). All statistical analyses were done using a statistical software package, SYSTAT. No corrections were done for multiple comparisons.

RESULTS

Gene frequency analyses and linkage disequilibrium

Table 3 presents the gene frequency estimates for each polymorphic site in the nondiabetic and diabetic groups, separately, along with the heterozygosity (H) and polymorphism information content (PIC) values. No significant difference was detected in allele frequencies among subgroups based on diabetic status, ethnicity, or sex (Tukey's multiple pairwise comparison test). The estimates of heterozygosity and the PIC values show that the HindIII and PvuII polymorphisms are moderately informative for association and linkage studies in these populations. Allele frequencies for both polymorphisms were in Hardy-Weinberg equilibrium in both the nondiabetic and the diabetic groups.

TABLE 3. Gene frequencies of polymorphic LPL sites in the diabetic and nondiabetic samples from the San Luis Valley, Colorado

Sample	HindIII			PvuII		
	(+) Allele[a]	H	PIC	(+) Allele[a]	H	PIC
Control						
NHW	0.74	0.38	0.31	0.55[b]	0.50	0.37
Hispanic	0.78	0.34	0.28	0.54	0.50	0.37
Diabetic						
NHW	0.74	0.39	0.31	0.59	0.49	0.37
Hispanic	0.80	0.32	0.27	0.55	0.50	0.37

Abbreviations: H, heterozygosity; PIC, polymorphic information content; NHW, non-Hispanic White.
[a]Presence of the restriction site.
[b]The PuvII polymorphism was not in Hardy-Weinberg equilibrium in NHW controls.

TABLE 4. Lipoprotein lipase haplotypes in the San Luis Valley population estimated by maximum likelihood methods and (by direct haplotype counts in singly heterozygous individuals)

Haplotype		Frequencies			
		Controls		NIDDM	
HindIII	PvuII	NHW	Hispanics	NHW	Hispanics
–	–	0.22 (0.13)	0.20 (0.11)	0.18 (0.12)	0.16 (0.12)
–	+	0.07 (0.07)	0.05 (0.05)	0.08 (0.08)	0.05 (0.05)
+	–	0.25 (0.32)	0.27 (0.35)	0.22 (0.26)	0.30 (0.35)
+	+	0.46 (0.48)	0.48 (0.50)	0.52 (0.54)	0.50 (0.52)
Total chromosomes		1030 (842)	710 (600)	150 (118)	382 (308)
Delta value[a]		0.365	0.377	0.363	0.329

[a] $P < 0.001$, χ^2 distribution, 1 d.f.

Table 4 presents the maximum likelihood estimates of the haplotype frequencies and the delta values reflecting the extent and significance of nonrandom associations between the two restriction sites. Also shown are the haplotype frequencies estimated by direct counts in individuals heterozygous for a single site. The two estimates are not significantly different. The delta values show a statistically significant association between the HindIII and the PvuII polymorphisms ($P < 0.001$) in all of the subgroups based on diabetic status and ethnicity. The relatively high PIC values of the two polymorphisms enable us to examine the impact of the LPL gene polymorphism on the quantitative variation of some metabolic traits.

Analyses of covariance in the nondiabetic group

Using ANCOVA, we tested the null hypothesis that mean quantitative trait levels (total cholesterol, LDL-C, HDL-C, triglycerides, fasting insulin, and fasting glucose) do not differ among genotypes. Since all the subjects in this study could not be unambiguously haplotyped, the effects of the genotypes on quantitative traits were examined separately for each restriction site. The analyses were carried out separately for categories of diabetic and nondiabetic status, ethnicity (Hispanics and NHW) and sex, as the levels of the quantitative traits under study vary between normoglycemic and diabetic individuals, ethnic groups, and sexes. Because of previous reports of variation in triglycerides among LPL genotypes, we sought to confirm or contradict this association in a large sample of normoglycemic individuals representative of the nondiabetic population of the San Luis Valley.

HindIII polymorphism

Table 5 summarizes the results of analysis of the quantitative trait variation with LPL/HindIII genotype in normoglycemic NHW and Hispanic males and females. Ethnicity has an effect only on mean fasting insulin levels with Hispanics having higher mean fasting insulin levels than NHWs. Sex has a significant effect on HDL cholesterol reflecting the usual finding that females have higher HDL-cholesterol levels than males in the general population. Higher HDL-cholesterol levels are seen in both Hispanic and NHW females. The lower fasting glucose levels in females probably reflect their slightly higher fasting insulin levels. Among the quantitative parameters examined, only triglyceride levels show genotype-specific effects after adjusting for significant concomitant variables. The genotype effect was highly significant after

TABLE 5. Age-, smoking-, and BMI-adjusted mean values of quantitative variables among LPL/HindIII genotypes by ethnicity and sex in the normoglycemic sample from the San Luis Valley

	Non-Hispanic White						Hispanic						*P* Value		
	Male			Female			Male			Female					
Variable	– / –	+ / –	+ / +	– / –	+ / –	+ / +	– / –	+ / –	+ / +	– / –	+ / –	+ / +	*Genotype*	*Sex*	*Ethnicity*
N	*15*	*98*	*137*	*21*	*102*	*158*	*10*	*57*	*118*	*10*	*69*	*117*			
Fast G (mg/dl)	*96.2*	*97.3*	*97.6*	*92.5*	*92.3*	*92.5*	*97.0*	*98.4*	*97.5*	*90.1*	*92.6*	*93.2*	*0.760*	< 0.001	0.465
Fast I (μIU/ml)	13.3	9.9	10.5	9.4	9.2	10.1	13.3	11.1	10.5	11.8	12.2	11.6	0.175	0.184	< 0.001
Tchol (mg/dl)	210.4	212.7	215.9	220.7	219.9	216.5	201.9	214.9	217.7	212.9	210.2	213.2	0.829	0.629	0.521
HDL-C (mg/dl)	44.6	43.9	42.7	57.0	58.0	53.2	43.3	45.7	43.6	49.5	52.9	50.5	0.005	< 0.001	0.192
LDL-C (mg/dl)	136.4	139.9	142.0	137.5	135.9	135.3	132.5	137.8	141.0	137.7	129.4	130.1	0.851	0.018	0.232
Tg (mg/dl)	118.0	125.2	140.1	104.4	110.4	123.0	125.0	131.7	147.9	115.0	115.2	135.8	< 0.001	0.001	0.019

TABLE 6. Age-, smoking-, and BMI-adjusted mean values of quantitative variables among LPL/PvuII genotypes by ethnicity and sex in the normoglycemic sample from the San Luis Valley

	Non-Hispanic White						Hispanic						*P* value		
	Male			Female			Male			Female					
Variable	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	Genotype	Sex	Ethnicity
N	55	113	80	68	117	90	40	86	53	44	77	58			
Fast G (mg/dl)	97.2	96.6	98.7	91.1	93.4	92.2	100.4	97.8	96.1	94.8	92.1	92.5	0.636	< 0.001	0.442
Fast I (μIU/ml)	11.0	10.2	10.3	9.6	9.8	9.4	13.0	11.0	9.8	11.6	12.0	11.7	0.125	0.366	< 0.001
Tchol (mg/dl)	215.1	208.0	219.8	219.9	221.0	213.6	211.4	217.6	219.5	225.7	205.6	210.4	0.331	0.532	0.725
HDL-C (mg/dl)	43.9	42.7	43.8	55.8	54.8	54.9	41.9	44.0	45.6	54.9	49.6	50.6	0.359	< 0.001	0.136
LDL-C (mg/dl)	142.4	135.4	144.4	136.4	137.6	133.3	134.4	140.6	144.1	139.7	126.3	128.7	0.525	0.032	0.388
Tg (mg/dl)	125.3	128.2	143.0	115.0	120.6	111.2	148.6	143.7	136.6	125.4	126.0	132.1	0.801	0.003	0.009

separating the sample on the basis of sex and ethnicity, both of which also have a significant impact on triglyceride levels. The (+/+) genotype is associated with higher triglyceride levels in males and females in both ethnic groups. In all categories, the heterozygous (+/−) genotype class has levels of triglycerides intermediate between the low (−/−) and high (+/+) homozygous genotype class. This gene dosage effect suggests a codominant mode of action of the associated variation. The average allelic excess associated with the (−) allele of the HindIII polymorphism is −12.85 mg/dl and −10.91 mg/dl in NHW and Hispanic males, respectively and −8.06 and −12.47 mg/dl in NHW and Hispanic females.

A significant association (P = 0.005) was also observed between HindIII genotype and HDL-cholesterol levels. The (+/+) genotype is consistently associated with lower levels of HDL-cholesterol although no clear gene dosage effect is seen.

PvuII polymorphism

Table 6 summarizes the results of analysis of quantitative trait levels with LPL/PvuII genotype in normoglycemic Hispanic and NHW males and females. Although the number of individuals included in this analysis (N = 881) was slightly smaller than that in the LPL/HindIII analysis (N = 912) because of differences in the number of unequivocal genotypes observed in the two systems, the pattern of sex and ethnicity effects on quantitative variables was the same. Unlike the HindIII analysis, there was no significant association between PvuII genotype and any quantitative trait.

Analyses of covariance in the diabetic group

The analysis of genotype specific effects in the diabetic group was complicated by the smaller number of diabetics compared to the normoglycemic sample, and the skewed allele frequencies at the test loci.

HindIII polymorphism

Table 7 presents the comparison of levels of quantitative traits among HindIII genotypes in the diabetic group by ethnicity and sex. Only fasting insulin level showed a significant difference between the (+/−) and (+/+) genotypes in Hispanic males (P = 0.009). Since the number of homozygotes for the absence of the restriction site was only two in Hispanic males, only the two common genotypes were included in the analysis [(+/−), 14.69 ± 1.82 μIU/ml; (+/+), 24.64 ± 2.51 μIU/ml]. This effect is significant only in Hispanic males and an examination of fasting insulin levels in other categories reveals no consis-

TABLE 7. Age-, smoking-, and BMI-adjusted mean values of quantitative variables among LPL/HindIII genotypes in NIDDM by ethnicity and sex

	Non-Hispanic White						Hispanic						*P* value		
	Male			Female			Male			Female					
Variable	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	Genotype	Sex	Ethnicity
N	6	11	26	1	14	17	2	26	47	7	34	75			
Fast G (mg/dl)	126.2	194.6	184.6	104.4	200.2	161.6	221.4	169.0	171.0	182.6	186.2	175.6	0.346	0.375	0.108
Fast I (μIU/ml)	28.5	22.5	16.6	11.5	30.9	22.7	27.2	14.7	24.6	31.9	22.3	23.0	0.653	0.711	0.453
Tchol (mg/dl)	203.2	216.0	199.4	194.0	207.0	210.4	225.1	212.9	214.0	227.9	248.3	234.5	0.650	0.295	0.038
HDL-C (mg/dl)	37.1	38.6	35.1	34.4	45.5	47.2	48.9	39.0	38.3	56.1	45.8	44.9	0.820	0.041	0.067
LDL-C (mg/dl)	129.4	144.6	121.7	129.5	120.3	122.6	152.9	134.9	129.1	129.7	157.0	139.3	0.621	0.879	0.149
Tg (mg/dl)	207.3	168.6	200.2	150.4	189.5	162.9	127.2	181.3	217.3	198.1	226.9	210.3	0.596	0.677	0.508

TABLE 8. Age-, smoking-, and BMI-adjusted mean values of quantitative variables among LPL/PvuII genotypes in NIDDM by ethnicity and sex

	Non-Hispanic White						Hispanic								
	Male			Female			Male			Female			*P* value		
Variable	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	−/−	+/−	+/+	Genotype	Sex	Ethnicity
N	9	14	21	4	23	5	15	37	23	27	55	37			
Fast G (mg/dl)	157.4	186.0	178.2	209.2	166.3	192.2	163.8	175.7	169.9	172.2	180.1	176.9	0.908	0.745	0.585
Fast I (μIU/ml)	21.6	17.9	19.6	40.4	21.9	33.6	16.6	23.6	18.3	25.9	23.4	20.1	0.467	0.180	0.392
Tchol (mg/dl)	209.4	222.8	190.5	211.1	205.7	218.5	212.5	214.9	213.1	247.0	238.1	231.9	0.952	< 0.001	0.061
HDL-C (mg/dl)	33.9	36.6	37.2	46.9	46.6	42.7	39.9	38.9	37.7	48.2	44.8	45.9	0.770	< 0.001	0.243
LDL-C (mg/dl)	137.4	150.4	113.4	120.3	120.7	129.5	125.8	133.3	133.7	144.5	142.5	144.8	0.836	0.108	0.223
Tg (mg/dl)	224.1	180.7	184.6	213.3	170.8	158.5	188.0	206.6	201.4	222.1	227.2	190.1	0.798	0.572	0.310

tent pattern of association (S. A. Cole, C. Aston, R. F. Hamman, and R. E. Ferrell, unpublished results). The association of the HindIII (+) allele with lower HDL cholesterol levels seen in the normoglycemic sample is also present in the NIDDM sample.

PvuII polymorphism

The results from the ANCOVA for the PvuII polymorphism among the diabetics, by ethnicity and sex, are shown in **Table 8.** Sex has effects on total cholesterol and HDL-cholesterol levels. Higher levels of total and HDL-cholesterol are seen in both Hispanic and NHW females. No significant association was detected between PvuII genotype and any quantitative trait.

DISCUSSION

In this study, no significant differences were seen in allele frequencies at the HindIII and PvuII polymorphic sites in the LPL gene between nondiabetics and diabetics or between NHW and Hispanics. Similar to previous findings in Caucasians and Japanese (16) our data also show a strong linkage disequilibrium between the HindIII and PvuII polymorphic sites in both Hispanics and non-Hispanic Whites. This significant disequilibrium is apparent whether one uses direct haplotype counts in individuals heterozygous for only a single site or uses the maximum likelihood estimate of haplotype frequencies in the whole sample.

The most significant finding in this study is the clear effect of the HindIII polymorphism in the LPL gene on the variation of plasma levels of triglycerides in normoglycemic sample. The (+) allele of the HindIII site is significantly associated with higher levels of triglycerides as compared to the (−) allele. There is a clear gene dosage effect with the heterozygotes having triglyceride levels intermediate between the two homozygous genotypes. No significant pattern of variation of triglyceride levels with PvuII genotype was observed. Among diabetics, there was no clear pattern of variation in triglyceride levels with either HindIII or PvuII genotype. This is not unexpected for several reasons. The number of diabetic individuals in each ethnicity × sex category was small and the gene frequencies for the HindIII polymorphism are skewed. Perhaps more important, NIDDM is known to have a significant impact on triglyceride levels and the magnitude of this effect depends on duration of disease and degree of metabolic control. In this sample, knowledge of duration of disease and information regarding metabolic control were not available.

These results confirm an earlier trend (S. A. Cole, C. Aston, R. F. Hamman, and R. E. Ferrell, unpublished results) and the work of Chamberlain et al. (16), who reported a strong association of the HindIII (+) allele with primary hypertriglyceridemia in Europeans and Japanese. The same group (17) has also noted a significantly high frequency of the (+) allele in patients with coronary atherosclerosis compared to controls. As LPL activity is known to affect HDL-cholesterol through its hydrolysis of triglyceride-rich lipoproteins (36, 37), it is possible that the HindIII (+) allele may be involved in affecting the LPL activity which in turn leads to hypertriglyceridemia and reduced HDL-cholesterol levels. In this regard, it is of interest that the (+/+) genotype, associated with higher triglyceride levels, is also associated with lower HDL-cholesterol levels in this normoglycemic population. This association is statistically significant ($P = 0.005$). The HindIII polymorphism is located in intron 8 in the LPL gene and does not cause a change in the sequence of the gene product. Thus any effects noted for this polymorphism must be due to its nonrandom association with a functional mutation elsewhere in the gene. In contrast to the work of Chamberlain et al. (16), who noted a significant effect of the PvuII polymorphism on triglyceride levels and its association with hypertriglyceridemia, we observed no significant impact of the PvuII polymorphism on triglyceride levels in this normoglycemic, normolipidemic population. The borderline effects of the PvuII polymorphism on triglyceride levels in

some categories reflects its nonrandom association with the HindIII variation. Similar to our present finding, an association between the HindIII polymorphism and plasma triglyceride levels has been noted in an Amerindian population (38).

An association between fasting insulin levels and the LPL/PvuII polymorphism in normoglycemic Hispanic males, with higher insulin levels being associated with the (−) allele (S. A. Cole, C. Aston, R. F. Hamman, and R. E. Ferrell, unpublished results) has been observed. In the present study, with a larger sample drawn from the same population, the same trend was observed in normoglycemic Hispanic and NHW males, but the genotype effects were not statistically significant. In rat adipocytes, both messenger RNA levels (4) and lipoprotein lipase activity levels (5, 39) have been shown to be influenced by insulin. Given the physiological link between insulin levels and lipoprotein lipase activity, further exploration of the possible relationship between genetic variation at the LPL locus and insulin levels is warranted.

The primary goal of this work was to test the associations between LPL gene variation and triglyceride and insulin levels in Hispanics (S. A. Cole, C. Aston, R. F. Hamman, and R. E. Ferrell, unpublished results) and the associations between LPL variation and triglyceride levels previously reported by others (12, 16, 38). We have confirmed a significant association between the LPL/HindIII polymorphism and triglyceride levels in normoglycemic males and females of Hispanic and non-Hispanic Caucasian origin in the San Luis Valley, CO. In addition, we have tested for associations between two LPL polymorphisms and a number of quantitative traits associated with lipid and glucose metabolism. No consistent significant association with these other variables was observed. We have not made adjustments for multiple comparisons in this study. While such adjustment reduces the frequency of type I error, it also increases the frequency of type II error. Thus, adjusting for multiple comparisons increases the likelihood of rejecting a significant association when one actually exists. As proposed by Rothman (40) we prefer to avoid adjusting for multiple comparisons in initial studies and rely on replication to eliminate spurious associations.

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