Lipoprotein Lipase Is a Gene for Insulin Resistance in Mexican Americans

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The insulin resistance syndrome is increasingly recognized as a risk factor for cardiovascular disease. Lipoprotein lipase (*LPL*) is a candidate gene for components of the syndrome. A small number of studies have demonstrated association of single nucleotide polymorphisms within *LPL* and indirect or surrogate measures of insulin resistance, largely based on glucose and insulin values obtained in the fasting state or during an oral glucose tolerance test. To test directly whether *LPL* is an insulin resistance gene, we performed the hyperinsulinemic-euglycemic clamp in a large family-based population of Mexican Americans who were genotyped at six polymorphisms in *LPL* that define the most common haplotypes in the population. *LPL* haplotypes showed linkage to the glucose infusion rate (GINF), a direct physiologic measurement of insulin sensitivity ($P = 0.034$). In addition, significant associations with GINF were demonstrated for the most common haplotype ($P = 0.031$) and the fourth most common haplotype ($P = 0.007$). Haplotype 1 was associated with insulin sensitivity (mean GINF for haplotype 1 carriers = 383.0 mg/min) and haplotype 4 with insulin resistance (mean GINF for haplotype 4 carriers = 344.3 mg/min). This haplotype-based genetic analysis provides compelling evidence that variation in the *LPL* gene plays a role in determining insulin resistance in this ethnic group with a high prevalence of the insulin resistance syndrome. *Diabetes* 53:214–220, 2004

The insulin resistance syndrome (also called the metabolic syndrome) is a clustering of factors associated with an increased risk of coronary heart disease (CHD) (1). The syndrome affects >20% of adults in the U.S., with the highest age-specific prevalence rates in Mexican Americans (2). Insulin resistance, whether accompanied by other features of the metabolic syndrome, has been associated with an increased risk of cardiovascular events and death (3,4). Given the association of insulin resistance and CHD, attention has been turned to identifying genetic determinants underlying the insulin resistance syndrome.

The lipoprotein lipase (*LPL*) gene has emerged as a candidate gene for features of the metabolic syndrome. Studies have identified linkage and association of the *LPL* gene with hypertension (5,6), indirect or surrogate measurements of insulin resistance (7,8), dyslipidemia (7,9–11), obesity (11), and atherosclerosis (12–14). LPL is an excellent candidate connecting insulin resistance to atherosclerosis because it controls the delivery of free fatty acids (FFAs) to muscle, adipose tissue, and vascular wall macrophages, wherein lipid uptake influences peripheral insulin sensitivity, central obesity, and foam cell formation (15).

We have genotyped a series of *LPL* 3′ end single nucleotide polymorphisms (SNPs) to determine the haplotype structure of this region of the *LPL* gene in the Mexican-American population. Our efforts are focused on the 3′ end of the *LPL* gene because polymorphisms in the 3′ end, such as *Hind*III, have been associated with surrogate measures of insulin resistance and atherosclerosis (7,8,13,14). To date, each of several published reports of positive linkage or association of variation in *LPL* with indexes of insulin sensitivity examined only one or two SNPs (7,8,16–21). Recent studies suggest that the extensive variation in human beings is best described by groups of associated polymorphisms referred to as haplotypes (22,23). Haplotypes encompass chromosomal blocks that have remained unbroken by recombination during the population evolutionary history of the gene. Haplotypes are more likely to identify disease associations than single polymorphisms because they reflect global gene structure and encompass the majority of common variation in a gene. Identification of a haplotype associated with increased or decreased disease risk should facilitate identification of the actual functional variant that affects disease risk because this variant should lie on chromosome regions identified by that haplotype (24). Using a haplotype-based analysis, we recently demonstrated association of *LPL* 3′ end haplotypes with coronary artery disease (CAD) in Mexican Americans (14). Our goal herein was to use these haplotypes to explore association of variation in *LPL* with insulin resistance.

Besides examining only one variant in the *LPL* gene, prior studies suggesting that *LPL* influences insulin sensitivity often used only surrogate measurements of insulin

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CAD, coronary artery disease; CHD, coronary heart disease; FFA, free fatty acid; GINF, glucose infusion rate; HOMA, homeostasis model assessment; LPL, lipoprotein lipase; MACAD, Mexican-American Coronary Artery Disease; SNP, single nucleotide polymorphism.

TABLE 1
LPL single marker and haplotype frequencies in Mexican Americans

SNPs and major allele frequencies	7315 G→C 0.89	8292 A→C 0.85	8393 T→G 0.80	8852 T→G 0.78	9040 C→G 0.93	9712 G→A 0.88	Chromosomes	Frequency (%)
Haplotype 1	G	A	T	T	C	G	206	62.8
Haplotype 2	G	C	T	T	C	G	50	15.2
Haplotype 3	C	A	G	G	C	A	33	10.1
Haplotype 4	G	A	G	G	G	G	22	6.7
Haplotype 5	G	A	G	G	C	A	8	2.4
Haplotype 6	G	A	T	G	C	G	6	1.8
Haplotype 7	C	A	G	G	C	G	2	0.5
Haplotype 8	G	A	G	G	C	G	1	0.3

sensitivity, typically based on blood taken in the fasting state or during an oral glucose tolerance test (7,16–21). Thus, current evidence that variation in *LPL* plays a role in insulin sensitivity is indirect. Assessment of the glucose infusion rate (GINF) during the hyperinsulinemic-euglycemic clamp study is widely regarded as the most direct physiologic measurement of insulin sensitivity (25). To address this issue directly, in this study we assessed insulin sensitivity by the euglycemic clamp as the phenotype for linkage and association. In addition, we used haplotypes rather than a SNP. Our results provide direct evidence that *LPL* is an insulin resistance gene by demonstration of both linkage and association of *LPL* haplotypes with a direct quantitative measurement of insulin resistance in Mexican-American families.

RESEARCH DESIGN AND METHODS

The UCLA/Cedars-Sinai Mexican-American Coronary Artery Disease (MACAD) project enrolls families ascertained through a proband with CAD, determined by evidence of myocardial infarction on electrocardiogram or hospital record, evidence of atherosclerosis on coronary angiography, or history of coronary artery bypass graft or angioplasty (14). Two generations are enrolled in the study: *1*) the proband and proband spouses (parental generation); and *2*) their adult (aged ≥18 years) offspring and the spouses of those offspring (offspring generation). DNA was obtained for genotyping from members of both generations, and only members of the offspring generation were asked to undergo a series of tests to characterize their metabolic and cardiovascular phenotype.

All studies were approved by Human Subjects Protection Institutional Review Boards at UCLA and Cedars-Sinai Medical Center. All subjects gave informed consent before participation.

Genotyping. In a prior study, we determined a set of six SNPs that are sufficient to identify the most common haplotypes occurring in the 3′ end of the *LPL* gene (14). These are 7315, 8292, 8393, 8852, 9040, and 9712. The numbering of the SNPs corresponds to Genbank accession no. AF050163, which describes a 9.7-kb segment of the *LPL* gene originally sequenced in the MDECODE (Molecular Diversity and Epidemiology of Common Disease) project, a study of Finns, non-Hispanic Caucasian Americans, and African-American subjects (26). SNP 8393 is the *Hind*III variant located in intron 8, and 9040 is the Ser447Stop variant located in exon 9. SNP 7315 is in intron 7, 8292 and 8852 are in intron 8, and 9712 is in intron 9. Large-scale genotyping of the six SNPs in MACAD families was performed using the 5′-exonuclease (Taqman MGB) assay (27). A description of this technique and PCR primer and oligonucleotide probe sequences is given in Goodarzi et al. (14).

LPL markers were genotyped in 514 individuals from 85 MACAD families. Of these, 29 genotyped individuals were discarded because their genotypes were incompatible with their family pedigree, as detected by the program PedCheck (28). This left 485 individuals from 80 families genotyped at *LPL*. The genotype frequencies for all six markers were in Hardy-Weinberg equilibrium. Linkage disequilibrium among the six markers (D′) ranged from 0.46 to 0.87 (29).

Phenotyping. The adult offspring of the proband and the spouses of the offspring undergo a 3-day phenotyping protocol, which includes indexes of insulin resistance determined by euglycemic clamp. Of the 485 subjects genotyped at *LPL*, 125 were from the parental/proband generation that was not phenotyped, and 69 (from six families) from the offspring generation were not clamped. Thus, 291 subjects from 74 families were both clamped and genotyped for the *LPL* markers.

Several indexes of insulin sensitivity are obtained in the MACAD study. Fasting insulin and glucose, themselves simple surrogate measures of insulin sensitivity, allow calculation of the homeostasis model assessment (HOMA) index. Using glucose in mmol/l and insulin in μIU/ml, the HOMA index is calculated as (glucose × insulin)/22.5. An ideal, normal-weight person aged <35 years has a HOMA of 1 (30).

During the hyperinsulinemic-euglycemic clamp (25), a priming dose of human insulin (Novolin; Novo Nordisk, Clayton, NC) was given and followed by infusion for 120 min at a constant rate (60 $mU \cdot m^{-2} \cdot min^{-1}$) to achieve a plasma insulin concentration of ≥100 μIU/ml. Blood was sampled every 5 min, and the rate of 20% dextrose coinfused was adjusted to maintain plasma glucose concentrations at 95–100 mg/dl. The GINF (given in mg/min) over the last 30 min of steady-state insulin and glucose concentrations reflects glucose uptake by all tissues of the body (primarily insulin-mediated glucose uptake in muscle) and is therefore a direct physiologic measurement of tissue insulin sensitivity. GINF is also often reported divided by body weight, resulting in a trait termed the *M* value ($mg \cdot kg^{-1} \cdot min^{-1}$) (25). GINF and the *M* value underestimate the total glucose disposal rate during the euglycemic clamp in conditions where hepatic glucose output is not completely suppressed by the insulin infusion. In nondiabetic insulin-resistant subjects, such as those in our study, *M* underestimates total glucose disposal only by ≤10% (31).

Data analysis. Based on the pedigree structures and genotype data of all individuals in each pedigree, haplotypes were constructed as the most likely set (determined by the maximum likelihood method) of fully determined parental haplotypes of the marker loci for each individual in the pedigree, using the simulated annealing algorithm implemented in the program Simwalk2 (32). Using this method, we were able to assign haplotypes to 475 of the 485 genotyped subjects, including 284 of the 291 genotyped and clamped subjects, comprising 199 offspring and 85 offspring spouses. Founder haplotypes, i.e., those haplotypes from parents (48 spouses of probands) and individuals marrying into the families (116 spouses of offspring), were used to calculate haplotype frequencies in 328 chromosomes from 164 Mexican-American founders without CAD. The frequencies of the most common haplotypes are displayed in Table 1 along with the major allele frequencies of the six SNPs. The markers from Mexican Americans without CAD were used for haplotype frequency estimation in order to eliminate any disease-based ascertainment bias.

Log-transformed (anthropometric measurements, fasting glucose, and fasting insulin) or square root–transformed (HOMA, GINF, and *M*) trait values were used to reduce skewness for all statistical analyses. Unpaired, two-sided *t* tests were used to compare trait values between men and women.

Linkage was assessed using sibpair analysis (33). The basic idea of this approach is that if a locus influences the quantitative trait or phenotype under study, then siblings who share more alleles at that locus will be more similar in phenotype than siblings who share fewer alleles. Conceptually, this procedure first plots the square of the difference in the quantitative trait between each sibpair versus the number of alleles shared and then uses linear regression to estimate how much of the difference in the trait depends on the number of alleles shared. A significant linkage is shown by a negative regression coefficient. If there is no linkage, the regression coefficient is expected to be zero. We used the SIBPAL2 program in SAGE 4.2 (34) to implement a sibpair analysis that uses the mean-corrected cross-product instead of the squared difference of the sibs' trait values as the dependent variable; this revised method has more power and accommodates multiple sibs in a family (35). Age, sex, and BMI were specified as covariates in the

TABLE 2
Clinical characteristics of 291 genotyped and clamped individuals

	Men	Women
n	112	179
Age (years)	35 ± 9.4 (19–60)	35.5 ± 8.2 (18–58)
Weight (kg)*	84.2 ± 15.6 (52.5–126.6)	72.1 ± 14.0 (38.6–128.5)
Body mass index (kg/m^2)	28.9 ± 4.8 (17.8–45.4)	29.1 ± 5.5 (18.1–54.8)
Fasting glucose (mg/dl)†	96.1 ± 9.8 (74.0–118.0)	92.5 ± 9.4 (56.0–117.0)
Fasting insulin (μlU/ml)	15.4 ± 8.9 (5.0–62.0)	15.5 ± 7.5 (2.0–49.0)
HOMA	3.7 ± 2.4 (1.2–15.9)	3.6 ± 1.9 (0.5–14.0)
GINF (mg/min)‡	428.6 ± 196.8 (105.9–1031.5)	343.5 ± 147.5 (20.7–1010.5)
M (mg · kg^{-1} · min^{-1})	5.4 ± 2.8 (1.0–13.9)	5.0 ± 2.4 (0.2–14.9)

Data are mean ± SD (range). Comparing men versus women, *$P < 0.00001$, †$P = 0.005$, ‡$P = 0.0001$.

linkage analysis. Among our subjects who were genotyped and clamped, we had available 252 sibpairs for linkage analysis.

Association was evaluated by quantitative transmission disequilibrium testing for both individual polymorphisms and haplotypes using the QTDT program (36). The transmission disequilibrium test was first developed for dichotomous traits in which alleles transmitted and not transmitted from the parents to affected offspring are compared to determine whether one allele is associated with the disease in question (37). It has the desired property of not giving spurious significant results attributable to population stratification because both case and control alleles come from the same parents. The transmission disequilibrium test was later extended to quantitative traits (38). Abecasis, Cardon, and Cookson (36) developed a general approach for scoring allelic transmission that accommodates families of any size and uses all available genotypic information. Family data allow the construction of an expected genotype for every nonfounder, and orthogonal deviates from this expectation are a measure of allelic transmission. The QTDT program implements this general transmission disequilibrium testing using the orthogonal model of Abecasis, Cardon, and Cookson (39). Age, sex, and BMI were specified as covariates. Environmental variance, polygenic variance, and additive major locus were specified in the variance model.

RESULTS

The clinical characteristics of the 291 subjects (112 men and 179 women) who had quantitative assessment of insulin resistance are shown in Table 2. This is an adult group of Mexican Americans of mean age 35.3 years. On average, these individuals are overweight. This may account for the degree of insulin resistance observed; however, it is known that Mexican Americans have a predisposition to visceral adiposity, hyperinsulinemia, and insulin resistance (40,41). The mean HOMA level suggests that these people are on average three to four times more insulin resistant than normal. The men had statistically significantly higher weight ($P < 0.00001$) and fasting glucose ($P = 0.005$) levels, while the women had significantly lower GINF ($P = 0.0001$) but not *M* values. These differences remained significant among the 284 subjects who were clamped and haplotyped.

Of the several indexes of insulin sensitivity, linkage with *LPL* haplotypes was demonstrated only for the direct quantification represented by GINF ($P = 0.034$). The *M* value, a clamp-derived index equal to GINF divided by body weight, was not significantly linked to *LPL* haplotypes ($P = 0.32$). All other measures (fasting glucose, fasting insulin, and HOMA) were not significant ($P = 0.82$, 0.44, and 0.34, respectively).

Association was evaluated by quantitative transmission disequilibrium testing. No haplotype was significantly associated with fasting glucose, fasting insulin, or HOMA, but both haplotypes 1 and 4 were significantly associated with GINF (haplotype 1, $P = 0.031$; haplotype 4, $P = 0.007$) and the *M* value (haplotype 1, $P = 0.031$; haplotype 4, $P = 0.005$). To characterize the nature of the associations of haplotypes 1 and 4 with insulin resistance, we determined the mean levels of insulin sensitivity in carriers of these haplotypes (Table 3 and Fig. 1). We observed that haplotype 1 is associated with the most favorable mean insulin sensitivity, while carriers of haplotype 4 had the lowest insulin sensitivity (i.e., the greatest insulin resistance). For fasting insulin, HOMA, GINF, and *M*, mean insulin sensitivity progressively deteriorated, going from haplotype 1 homozygotes to haplotype 1 heterozygotes to individuals without haplotype 1. Conversely, haplotype 4 heterozygotes were more insulin resistant than those without haplotype 4 (no haplotype 4 homozygotes were observed among the clamped subjects). Figure 2 further explores these associations by independently examining the effects of haplotypes 1 and 4 on insulin sensitivity. Exclusion of subjects with haplotype 4 from haplotype 1 heterozygotes and those without haplotype 1 did not affect the trend of benefit on insulin sensitivity seen with increasing numbers of haplotype 1. Similarly, excluding haplotype 1 carriers from those without and with haplotype 4 did not affect the trend of lower insulin sensitivity in the latter subjects; in fact, the subjects without haplotype 1 who were carriers of haplotype 4 had the lowest insulin sensitivity (most insulin resistance) compared with the other haplogenotype groups. Similar trends were observed with *M* value (data not shown).

DISCUSSION

LPL haplotypes showed both linkage and association with insulin sensitivity in this study of Mexican Americans ascertained via a parent with CAD. The strength of this investigation is that we examined a population at high risk for the insulin resistance syndrome on clinical genetic epidemiologic grounds, that we directly quantified insulin sensitivity by the euglycemic clamp study, and that we

TABLE 3
LPL haplotype means for indexes of insulin sensitivity

Haplotype carriers	Fasting glucose (mg/dl)	Fasting insulin (μlU/ml)	HOMA	GINF (mg/min)	*M* (mg · kg^{-1} · min^{-1})
1 ($n = 239$)	93.8	14.0	3.0	383.0*	5.3*
2 ($n = 88$)	95.2	14.6	3.1	365.9	4.9
3 ($n = 56$)	94.9	14.5	3.1	345.1	4.7
4 ($n = 40$)	93.3	15.5	3.2	344.3*	4.6*

*Significant association of phenotype with haplotype (see text).

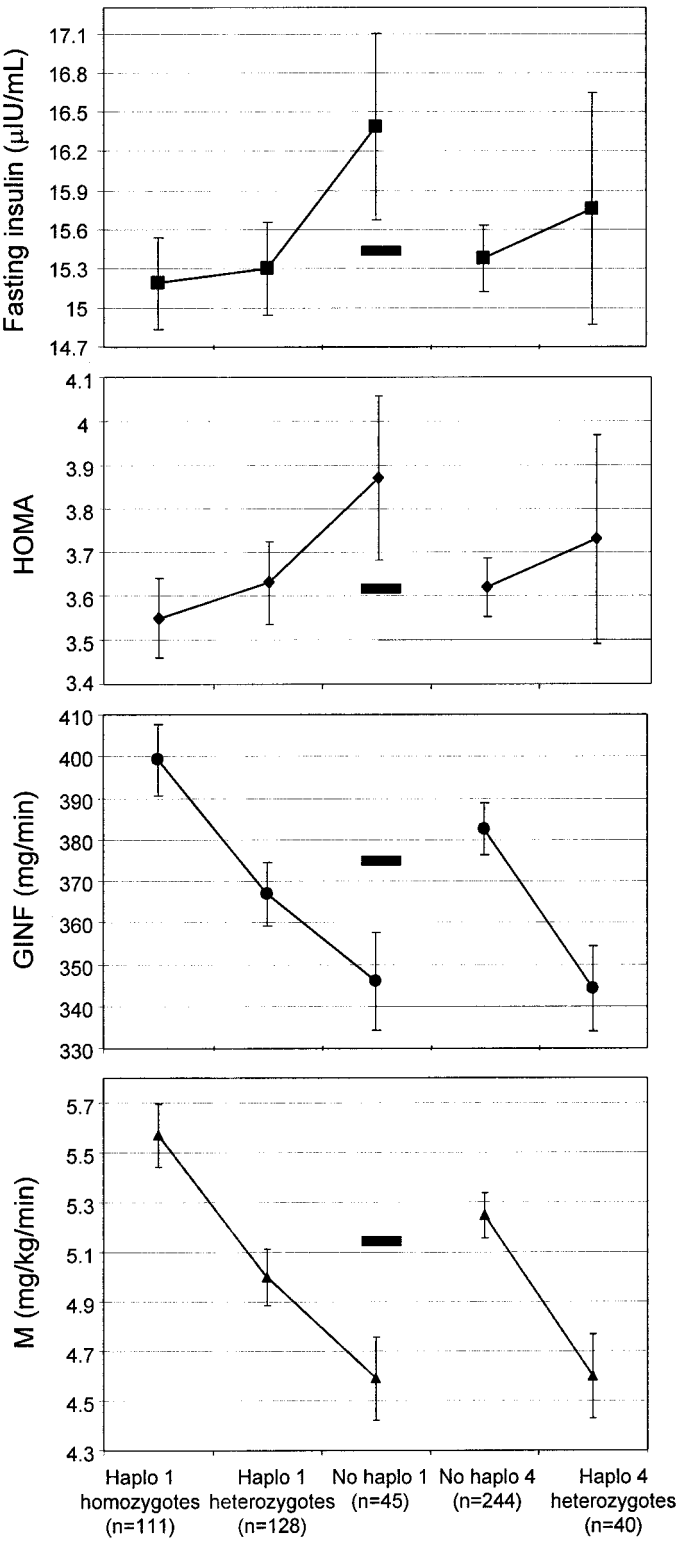

FIG. 1. Effect of *LPL* 3′ end haplotypes on indexes of insulin sensitivity. Each point represents the mean trait value for the haplogenotype indicated at the bottom. The thick line in the center of each graph represents the mean for the entire haplotyped and clamped population. Vertical lines represent SE.

combined this with the power of a haplotype-based analysis. The results suggest the presence of a common *LPL* haplotype in this population that protects against insulin resistance and a common haplotype that predisposes to insulin resistance. Of interest, our prior work indicated that these same *LPL* haplotypes appear to protect and predispose, respectively, to clinical CAD (14).

The clustering of insulin resistance, hypertension, central obesity, and dyslipidemia in the metabolic syndrome is receiving much attention as a risk factor for cardiovascular disease. The central component of this syndrome, insulin resistance, has been found to increase cardiovascular risk. In the San Antonio Heart Study, insulin resistance, estimated by HOMA, was an independent predictor of incident cardiovascular events over 8 years of follow-up (4). In the Helsinki Policemen Study, 970 men free of diabetes or CHD at baseline were followed for 22 years; those with the highest levels of insulin resistance, as estimated by insulin area under the curve during oral glucose tolerance testing, had the highest rates of CHD events and death (3).

Our group is studying the *LPL* gene as a candidate gene for the insulin resistance syndrome. LPL hydrolyzes triglycerides carried in chylomicrons and VLDLs, the rate-limiting step in delivery of FFAs to muscle and adipose tissue. By controlling the delivery of FFA to muscle, LPL may affect insulin sensitivity by influencing levels of intramyocellular lipid, which correlate with muscle insulin resistance (42). In fact, transgenic mice with muscle-specific LPL overexpression exhibit whole-body and muscle insulin resistance (43). Conversely, in humans, a positive correlation between GINF and skeletal muscle LPL activity has been observed (44). Also, LPL may influence insulin resistance by affecting FFA delivery to visceral adipose tissue, which is increasingly viewed as an endocrine organ capable of secreting mediators of insulin resistance (45). LPL action also regulates the plasma triglyceride concentration, an important atherosclerosis risk factor (46). LPL activity indirectly raises HDL cholesterol levels because LPL-mediated hydrolysis of VLDL provides surface components that merge with HDL3 to form HDL2 particles (47). LPL-mediated delivery of FFA and lipoprotein remnants to vessel wall macrophages plays a role in foam cell formation, an early event in the development of atherosclerotic plaque (15). This atherogenic role of LPL is supported by studies wherein macrophages from LPL knockout mice prevented atherosclerosis when transplanted into irradiated atherosclerosis-prone LDL receptor knockout mice (48). Thus, functional variation in LPL may impact both insulin resistance and atherosclerosis.

LPL undergoes complex, tissue-specific regulation; for example, in the fed state, adipose LPL activity is increased and muscle LPL activity depressed, whereas the reverse is true in the fasting state (49). In insulin resistance/diabetes, macrophage LPL activity is increased and adipose LPL is decreased, with both alterations possibly contributing to atherosclerosis (15). The *LPL* haplotypes we have studied may contain variants that alter disease risk by affecting tissue-specific regulation of LPL activity. For example, one possibility is that haplotype 4 is associated with increased activity of LPL in muscle (promoting insulin resistance) and in macrophages (predisposing to atherosclerosis).

Most studies that have reported association of the *LPL* gene with insulin resistance used only surrogate measurements of insulin resistance, including fasting glucose

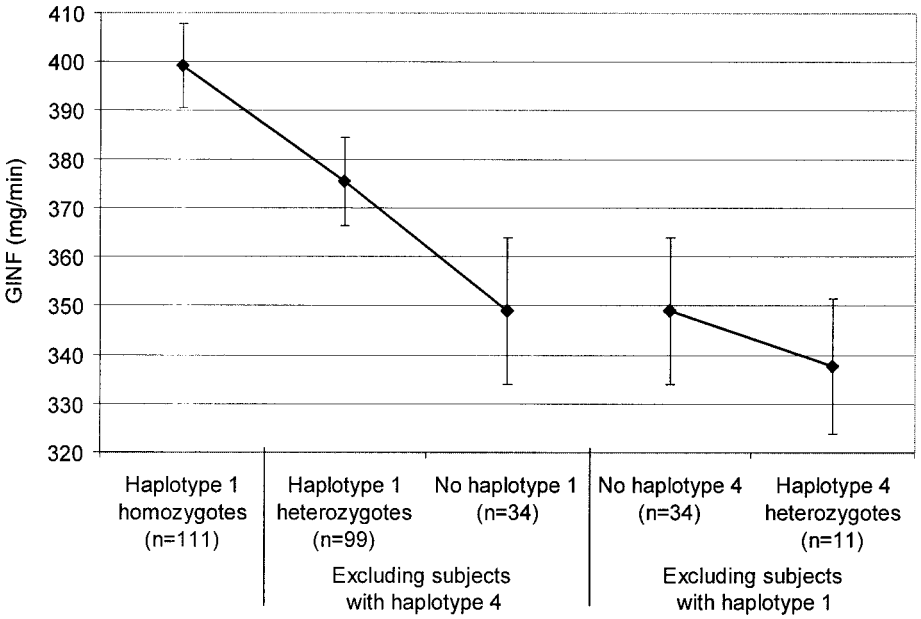

FIG. 2. Independent effects of haplotype 1 and haplotype 4 on insulin sensitivity. *Left*: Haplotype 1 genotypes with haplotype 4 carriers removed. *Right*: Haplotype 4 genotypes with haplotype 1 carriers removed. Each point represents the mean GINF value for each haplogenotype group. Vertical lines represent SE.

(16,17), fasting insulin (7,18–20), and insulin area under the curve during oral glucose tolerance testing (21). One study evaluated the steady-state plasma glucose during the insulin suppression test (8). In addition, all except one (20) of these studies only examined the association of the intronic restriction fragment–length polymorphisms *Pvu*II and *Hind*III. Thus, current evidence that variation in *LPL* plays a role in insulin sensitivity has been indirect. Assessment of GINF during the hyperinsulinemic-euglycemic clamp study is widely regarded as the most direct physiologic measurement of insulin sensitivity (25). An analysis of indexes of insulin sensitivity in the Insulin Resistance Atherosclerosis Family Study showed that direct physiologic measurements of insulin sensitivity have a higher heritability than measures based on fasting values (such as HOMA) (50). Thus, use of physiologic indexes rather than simple fasting indexes should provide more power to discover genes that contribute to insulin sensitivity. Our study is the first that has used insulin sensitivity assessed by the euglycemic clamp as the phenotype in an association study with *LPL*. Consistent with the described higher heritability of physiologic indexes over fasting indexes, we showed a statistically significant association of *LPL* with GINF and *M* value but not with fasting glucose, fasting insulin, or HOMA. In addition, this study contains one of the largest family cohorts in the literature that have undergone the euglycemic clamp. We thus provide here statistical genetic evidence that *LPL* is an insulin resistance gene by demonstration of both linkage and association of *LPL* haplotypes with a direct quantitative measurement of insulin resistance in Mexican-American families ascertained via CAD. Whether these *LPL* haplotypes have the same relationship with insulin resistance in Mexican Americans without a family history of CAD or in other ethnic groups is unknown.

Two *LPL* haplotypes were associated with variation in GINF. These haplotypes had opposite effects on insulin sensitivity. Haplotype 1, the most common haplotype, was associated with improved insulin sensitivity. As the number of chromosomes in an individual with haplotype 1 decreased (from two, to one, to none), insulin sensitivity by GINF, as well as HOMA and fasting insulin, decreased progressively. Furthermore, haplotype 4 carriers had the lowest insulin sensitivity, i.e., they were the most insulin resistant. The direction of these associations persisted when the haplotypes were considered separately. The available data indicate that there is an insulin-sensitizing functional variant on haplotype 1 chromosomes and a variant on haplotype 4–bearing chromosomes that promotes insulin resistance. Current data does not allow us to distinguish whether the actual nucleotide locus responsible is the same for both haplotypes or whether such variation is at different locations in the gene. In terms of the relation to cardiovascular risk associated with the metabolic syndrome, our previous work has shown that haplotype 1 is associated with protection against CAD and that haplotype 4 may be associated with increased risk of CAD (14).

The benefit of a haplotype-based analysis is that it captures all of the variation across a region, which should improve the ability to detect an association. To our knowledge, we are the first to apply the haplotype approach to the study of *LPL* as a gene influencing insulin sensitivity. All prior published studies reporting association of the *LPL* gene with insulin resistance used only single variants, usually *Hind*III or *Pvu*II (7,8,16–21). In some cases, the results are in conflict; studies have reported the T allele of *Hind*III associated with insulin resistance (7), others report the G allele associated with insulin resistance (8,21), and others show no association of *Hind*III with insulin resistance (16). This demonstrates a limitation of the common approach of examining one or two polymorphisms per candidate gene in an association study. By identifying whole chromosomal regions, haplotypes should have improved power and reproducibility in elucidation of disease-gene associations.

Multiple testing is an issue that applies to all genetic studies in which multiple genetic variants are tested for association against multiple traits. In such studies, including ours, adjusting for multiple comparisons by such

methods as Bonferroni corrections are typically not utilized because they result in a significance level that is too stringent, particularly for detection of associations of moderate genetic effects. The principal reason we did not adjust for multiple testing is that our goal was to test the prior hypothesis that *LPL* haplotypes are associated with the most direct measure of insulin sensitivity, that defined by the euglycemic clamp. Upon finding significant association of *LPL* haplotypes with GINF, we then explored the associations with the other indexes of insulin sensitivity. The consistency of the trends in measures of insulin sensitivity in relation to the *LPL* haplotypes (Fig. 1) supports our association results.

In the study herein, a haplotype-based approach successfully identified linkage and association of variation in the *LPL* gene with a direct physiologic measurement of insulin sensitivity in Mexican Americans, providing strong evidence that *LPL* is an insulin resistance gene. Given prior work demonstrating association of single variants with atherosclerosis, dyslipidemia, obesity, and hypertension, the haplotypes described here should be used in future studies exploring the association of the *LPL* gene with components of the insulin resistance syndrome, especially in the Mexican-American population.

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