Insulin and 2-Hour Glucose Levels Are Inversely Related to Lp(a) Concentrations Controlled for LPA Genotype

David L. Rainwater, Steven M. Haffner

Abstract—In this study we assessed the relationship of lipoprotein(a) [Lp(a)] with diabetes status and with measures of glucose and insulin in a population of Mexican Americans having a high prevalence of non–insulin-dependent diabetes mellitus (NIDDM). Because of enormous allelic diversity at LPA [the locus encoding the apo(a) protein] that directly influences Lp(a) concentrations, it was first necessary to adjust for the large effects of variation at LPA. We calculated residual Lp(a) concentration as the difference between observed and expected; expected Lp(a) concentration was based on information from all family members sharing each identical-by-descent (IBD) allele. We found significant effects of sex and age on residual Lp(a) concentrations that increased with age (P = 0.0004) and in females (P = 0.0034). Although diabetes status per se was not related to residual Lp(a) concentrations (P = 0.097), we found that residual Lp(a) concentrations were inversely correlated with fasting insulin (P = 0.0017) and with insulin (P = 0.0028) and glucose (P = 0.0429) concentrations measured 2 hours after a glucose challenge. Furthermore, significant inverse correlations with the 2 insulin measures were observed for a subgroup of nondiabetic individuals. Inclusion of 2 lipid measures (plasma concentrations of cholesterol and triglycerides) in the models showed that the correlations with insulin and glucose were independent of the relationship between Lp(a) concentrations and the lipid measures. Also, we determined the residual size for each apo(a) isoform by adjusting for the IBD isoform group average. Although not related to diabetes status, residual apo(a) isoform size was positively correlated with fasting insulin (P = 0.0013) and with 2-hour glucose (P = 0.0246) and 2-hour insulin (P = 0.0182) concentrations. In addition, significant correlations for all 4 measures were found for the subgroup of nondiabetic individuals. Thus, the results demonstrate that glucose-intolerant individuals have significantly lower residual Lp(a) concentrations and a significant increase of residual apo(a) size.

Key Words: non–insulin-dependent diabetes mellitus ■ glucose tolerance ■ Lp(a) ■ apo(a) genotypes ■ Mexican Americans

Lipoprotein(a) [Lp(a)] is an unusual lipoprotein, a type of LDL that possesses the unique apolipoprotein apo(a). Lp(a) concentrations show considerable variation in normal, healthy individuals and are under strong genetic control.1–3 Lp(a) concentrations are regulated at the level of biosynthesis of the distinctive apo(a) protein that is encoded by the LPA locus.5 Interest in the control of Lp(a) concentrations stems from a number of studies, almost all of which report a strong relationship with cardiovascular disease.6 Despite the fact that nearly 90% of Lp(a) variation is controlled by variation at the locus encoding the apo(a) protein (LPA) there is, in fact, substantial within-genotype variation,7,8 which is due to non-LPA factors (eg, effects of environment and, potentially, other genes).

Identification of non-LPA factors, such as non–insulin-dependent diabetes mellitus (NIDDM), that might affect Lp(a) concentrations is made difficult by the enormous degree of allelic diversity at LPA. One group estimates that there are >100 alleles at LPA and that the heterozygosity index approaches 1,9 suggesting that the likelihood of 2 unrelated individuals having identical genotypes at LPA is low, even if they have similar apo(a) isoform phenotypes. The most prominent aspect of allelic diversity is a polymorphism for size of the apo(a) protein.10 This genetically specified variation predicts 40% to 70% of overall variation in Lp(a) concentrations.11 In addition, however, there are a number of reported sequence variants at LPA that may be associated with variation in Lp(a) concentrations12–17 and binding properties.18 This degree of genetic variation makes it important to control for LPA genotype before attempting to determine effects of environmental factors.19

A number of studies have investigated the possibility that diabetes may be 1 such non-LPA environmental factor that influences Lp(a) concentrations. It appears that IDDM, particularly with microalbuminuria, is associated with increased Lp(a) concentrations.20 More controversial20 is whether there might be a relationship between NIDDM and Lp(a) and consequently, whether associated alterations in Lp(a) may
help explain the 3-fold increased risk of cardiovascular disease for diabetic subjects. Our earlier studies found significantly lower Lp(a) concentrations in NIDDM subjects. In both studies, however, we adjusted Lp(a) concentrations for apo(a) size and thus, the genetic variance was only partially removed.

The relation of insulin levels to Lp(a) levels has been controversial. In 1 study, Lp(a) levels were lower in subjects with hyperinsulinemia, but in other studies, no significant relation of Lp(a) to insulin was found. However, apo(a) phenotypes were not determined in these studies. In a small study, in which apo(a) phenotypes were measured, Lp(a) levels were significantly related to insulin resistance (negative correlation) but not to insulin concentrations.

Thus, for the current study we devised a method to control for allelic variation at the LPA locus. The method exploits information from all family members who share a particular identical-by-descent (IBD) allele to generate an expected Lp(a) concentration or isoform size for that allele. We used this information to adjust Lp(a) concentrations for the genetic contributions by LPA to test the hypothesis that Lp(a) is inversely associated with 2 diabetes-related traits, insulin and glucose concentrations.

Methods

Subjects and Samples

Subjects in this study are participants in the San Antonio Family Heart Study, which is a family study concerned with risk factors for cardiovascular disease in Mexican Americans living in and around San Antonio, Tex. In total, 1418 individuals were enrolled in the study. At a clinic visit, information was obtained about a number of covariates (sex, age, medications, etc), and a fasting blood sample was obtained by venipuncture. Plasma was isolated by low-speed centrifugation, and aliquots were stored at 80°C in segments of plastic tubing, protected from oxidation and desiccation. In addition, an oral glucose tolerance test was administered, which involved blood sampling 2 hours after the subject consumed 75 g glucose and was obtained by venipuncture. Plasma was isolated by low-speed centrifugation, and aliquots were stored at 80°C in segments of plastic tubing, protected from oxidation and desiccation. In addition, an oral glucose tolerance test was administered, which involved blood sampling 2 hours after the subject consumed 75 g glucose.

Phenotypic Assessments

Diabetes was defined, by World Health Organization criteria, as satisfying at least 1 of the following: fasting glucose concentrations \( \geq 7.8 \text{ mmol/L} \) (\( \geq 140 \text{ mg/dL} \)), glucose concentration \( \geq 11.1 \text{ mmol/L} \) (\( \geq 200 \text{ mg/dL} \)) 2 hours after the glucose load, or taking medications for diabetes. All diabetic subjects in this study had NIDDM.

Biochemical Measurements

Plasma glucose concentrations were measured with an Abbott V/P analyzer, and serum insulin concentrations were determined by use of a commercially available radioimmunoassay kit (Diagnostic Products Corp). As indicated previously, coefficients of variation for these assays during the study were 6.5% for glucose and 8.0% for insulin. Plasma Lp(a) concentrations were measured by use of a “sandwich”-style ELISA (MacraLp(a), Strategic Diagnostics) with a BioTek EL340 microplate reader; the interassay coefficient of variation for control products run in this assay averaged 4.3%.

Estimation of Allele-Specific Lp(a) Concentration

Apo(a) isoforms in plasma were resolved by SDS electrophoresis in polyacrylamide gradient gels as described, transferred to nitrocellulose (BA83, Schleicher and Schuell) electrophoretically, and detected by immunological staining. Two primary antibodies directed against apo(a) were used: rabbit anti-baboon apo(a), which also binds the human proteins, and the monoclonal antibody 2D1 (a gift of PerImmune, Inc, Rockville, Md), which is believed to be directed against a nonrepetitive epitope of human apo(a). Allele-specific Lp(a) concentrations were estimated as follows: For double-banded phenotype samples, we estimated relative concentrations of isoforms by use of immunoblotting procedures with the monoclonal antibody 2D1. After color development and densitometry with an LKB Ultroscan laser densitometer, we measured relative amounts of stain in each isoform by curve-fitting procedures (PeakFit software, Jandel Scientific) as described. On average, 1.6 measurements (SD = 2.1) were used to estimate fraction of absorbance in each isoform band. Sample amounts loaded in each lane were adjusted to optimize the curve-fitting procedure. A survey of 632 accepted estimates showed that the mean amount loaded was 90 ng per band (MacraLp(a) assay value; median, 47 ng), and 95% of the loads were <330 ng. We calculated allele-specific Lp(a) concentration as the product of plasma Lp(a) concentration and fractional absorbance. Among single-banded phenotype samples, we included only samples that were known, on the basis of pedigree information, to be heterozygous-null (ie, heterozygous for the expressed allele and for a “null” allele whose protein product could not be detected in the plasma). In these heterozygous-null samples (n = 262), we assumed that the allele-specific concentration of the expressed allele equaled the plasma Lp(a) concentration and that the concentration of the null allele protein was zero. Samples from \( n = 500 \) individuals were eventually excluded from this study because either they had null/null phenotypes (n = 41) or single-banded phenotypes but could not be demonstrated to be heterozygous-null (n = 288), or they were excluded for a variety of technical reasons, such as pedigree inconsistencies, apo(a) phenotyping difficulties, and missing data (n = 180).

Determination of Residual Lp(a) Concentration

First, we calculated IBD allele group means. On the basis of pedigree information, we identified isoforms that were shared by 2 or more family members (ie, IBD) and for which we had allele-specific Lp(a) concentrations. IBD allele group means for all null alleles were assumed to be zero. For example, in a plasma sample there were 992 isoforms in 293 different IBD groups, we calculated group means by multifactor ANOVA, in which we also simultaneously adjusted for the significant effects of age (treated as a continuous variable) and sex (see the Results section). We found no significant difference in variances for residual Lp(a) concentrations for isoforms from double-banded versus heterozygous-null samples, suggesting that the method for estimating fractional absorbance did not generate additional error. Next, we identified those samples for which we had an IBD allele group mean Lp(a) concentration for each allele. Summing the 2 concentrations generates an expected plasma Lp(a) concentration, and subtraction of expected from observed generates sex- and age-adjusted residual Lp(a) concentration for each sample. In this manner, we have attempted to subtract the genetic contribution by the LPA locus; the residual should reflect non-LPA factors that influence Lp(a) concentrations. Altogether, we obtained estimates of residual Lp(a) concentrations for 473 samples, approximately one third of the starting number of samples.

Determination of Residual Apo(a) Size

Apo(a) sizes were estimated by comparison with mobilities of standard isoforms run in the adjacent lane as described previously. In samples from 864 individuals, we measured sizes of a total of 1288 apo(a) isoforms that could be assigned to an IBD isoform group (ie, shared by 2 or more family members; there were a number of isoforms with measures of size but no isoform-specific concentration measured above). We combined all acceptable observations so each isoform size measurement represented an average of 3.0 independent determinations (SD = 1.5) and the average coefficient of variation for this determination was 1.2%. To calculate residual apo(a) size, we first estimated mean size for each IBD isoform group and then...
subtracted that expected value from the size of the isoform estimated for each family member in the group.

Statistical Analyses
Statistical analyses were performed with a commercial package (StatGraphics Plus, Manugistics). In calculating the residual Lp(a) concentration, an arbitrary IBD isoform group number was included as a main factor in the ANOVA, along with sex and age (all 3 factors were significant at *P*<0.001). Because IBD isoform group membership accounts for the skewed distributions of Lp(a) in a population, it was not necessary to transform Lp(a) concentrations in this model. Thus, the residual for each expressed allele was adjusted for sex and age and for the large differences across allele groups.

**Results**

Validation of the Method for Estimating Fractional Absorbance
Reliability of the estimate for fractional absorbance (assessed in 158 samples, each run in replicate in different gels) was moderately high; repeatability was 0.967 and the coefficient of variation for the estimate of the smaller band was 7.06%. We assessed the linearity of raw peak area as a function of the amount of Lp(a) loaded in a lane. Figure 1 shows the results of 1 such experiment, for which the correlation coefficient was 0.98. Seven other experiments yielded similar results, with correlation coefficients ranging from 0.91 to 0.98 (mean was 0.94) and suggested that linearity extended to ≈400 ng per lane in this system.

Sex and Age Effects on Allele-Specific Lp(a) Concentrations
Allele-specific concentrations were estimated for expressed apo(a) isoforms as described in Methods. Figure 2A shows a frequency histogram for allele-specific concentrations, which was skewed toward lower concentrations and ranged from near 0 to >100 mg/dL. Among 992 expressed isoforms, there were 293 IBD allele groups for which we calculated an IBD group mean (an average of 3.4 persons sharing each IBD allele). IBD group explained ≈83% of the total variation in Lp(a) concentrations (ie, *h*^2^ = 0.83).

In addition, sex and age exerted significant effects on allele-specific Lp(a) concentrations: females had higher Lp(a) concentrations than did males (the difference was 1.03 mg/dL per isoform, *P*=0.0034, ANOVA), and Lp(a) concentrations also increased with age (0.043 mg/dL per year, *r*=0.13, *P*=0.00004, regression analysis). Therefore, in calculating IBD group means, we simultaneously adjusted allele-specific Lp(a) concentrations for both alleles.

Relationship of Diabetes Status and Residual Lp(a) Concentrations
We estimated residual Lp(a) concentrations for 473 subjects as described in Methods. Figure 2B shows the frequency histogram for residual Lp(a) concentrations in this population. Eighty-one of the subjects were diagnosed as diabetic. On average, diabetic subjects had lower Lp(a) concentrations than did nondiabetic subjects, but this difference was not statistically significant (*P*=0.097, Table 1).

Relationship of Residual Lp(a) With Insulin and Glucose Concentrations
To explore further the relationship with insulin resistance and glucose metabolism, we tested for correlation of residual Lp(a) with logarithmically transformed insulin and glucose concentrations. Table 2 shows that residual Lp(a) concentrations were significantly correlated with fasting and 2-hour insulin levels. Figure 3 shows a scatterplot for the relationship between fasting insulin and residual Lp(a) concentrations.

**Table 1. Effect of Diabetes Status on Residual Lp(a) Concentrations (mg/dL)**

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>392</td>
<td>−0.03±0.37</td>
</tr>
<tr>
<td>Diabetic</td>
<td>81</td>
<td>−1.53±0.81</td>
</tr>
<tr>
<td><em>P</em></td>
<td></td>
<td>0.097</td>
</tr>
</tbody>
</table>

*P* value taken from ANOVA.

**Figure 1.** Change in raw peak area (arbitrary units) as a function of different amounts of Lp(a) loaded in the lanes. Amount of Lp(a) in each apo(a) isoform band was varied by mixing 2 single-banded samples with known Lp(a) concentrations [MacraLp(a) values], and peak area was estimated by curve-fitting procedures. Correlation coefficient was 0.983 for the linear regression.

**Figure 2.** Frequency histograms for Lp(a) concentrations. A, Isoform-specific Lp(a) concentrations for 1572 expressed isoforms in the study. B, Residual Lp(a) concentrations (adjusted for age and sex) for 473 individuals for whom there were isoform-specific Lp(a) concentrations for both alleles.
The 2 insulin measures were significantly correlated with Lp(a), even in the subgroup of individuals who were nondiabetic (Table 2), but not for the subgroup of diabetic individuals (data not shown).

Among glucose measures, only 2-hour glucose levels were significantly correlated with residual Lp(a) concentrations for all subjects in this study (Table 2). However, neither fasting nor 2-hour glucose levels were correlated with residual Lp(a) concentrations in the subsets of nondiabetic subjects (Table 2) or diabetic subjects (data not shown). Stepwise regression analyses indicated that there was only 1 independent correlate of residual Lp(a) concentrations among the 4 measures of glucose and insulin, and that factor was fasting insulin for the entire group and 2-hour insulin for the nondiabetic subgroup (data not shown).

We analyzed these same relationships in models that also included 2 lipid metabolism–related measures, plasma concentrations of cholesterol and triglycerides, which were previously shown to be correlated with Lp(a).35 Inclusion of the 2 lipid measures did not abolish the significant correlations of the 5 diabetes-related traits (ie, fasting and 2-hour insulin and 2-hour glucose concentrations for all subjects and fasting and 2-hour insulin concentrations for nondiabetic subjects) with residual Lp(a) concentrations, despite the fact that the lipid measures also were strongly correlated ($P<0.0005$) with residual Lp(a) in every model (Table 3).

Similarly, stepwise regression analyses indicated that, in addition to the 2 lipid measures, there were significant, independent correlates of residual Lp(a) concentrations among the measures of glucose and insulin (2-hour insulin and fasting glucose for all subjects and 2-hour insulin for the subset of nondiabetic subjects; data not shown).

**Estimation of Residual Apo(a) Size**

Apo(a) size was determined by comparison with standards in an adjacent lane. As shown in Figure 4A, molecular weight estimates ranged from 400 000 to >900 000. We calculated the mean size for each IBD allele group, which was taken as the expected size. Residual apo(a) size was calculated as the difference between the observed isofrom size in each sample and the IBD allele group mean for that isofrom. Figure 4B shows the frequency histogram for residual apo(a) size in this population.

**Relationship of Apo(a) Isoform Size With Glucose and Insulin Concentrations**

There was no significant association of residual apo(a) isofrom size and diabetes status ($P=0.192$, data not shown). However, we did find significant, positive correlations of residual apo(a) isofrom size with fasting and 2-hour insulin and for 2-hour glucose levels (Table 4). Furthermore, when only the nondiabetic subgroup was considered, there were significant, positive correlations with all 4 measures of glucose and insulin. Figure 5 presents a scatterplot illustrating the positive correlation of residual apo(a) size with fasting insulin concentrations.

**Discussion**

Because variation in Lp(a) concentrations and apo(a) size is largely controlled by genetic variation at LPA, it has been difficult to demonstrate consistently that environmental factors, which influence variation in other lipoproteins, have any effects on Lp(a). These difficulties have led to uncertainty about whether Lp(a) concentrations can be modified by any nongenetic factor and, potentially, may have hampered development of therapies aimed at reducing the risk of cardiovascular disease owing to high concentrations of Lp(a). Many studies have attempted to adjust for genetic variation at LPA by including information about apo(a) isofrom size, which is inversely related to Lp(a) concentrations. However, there are many exceptions to this relationship, suggesting that control of apo(a) size is not the only aspect of genetic variation in Lp(a) exerted by LPA. Consequently, for this study we attempted to devise a method that would adjust Lp(a) concentrations for LPA genotype.

Our method for estimating the expected Lp(a) concentration for individuals in this study is complicated, and potentially, several artifacts could affect the results. Some have argued that an assay that uses anti-apo(a) as the detecting antibody (such as the one used in this study) may respond differently to different size isofroms.34,36 Thus, our assay might yield slightly higher Lp(a) concentrations for particles bearing larger apo(a) isofroms than for those bearing smaller isofroms. However, even if there are significant differences in assay response to specific isofroms, we do not expect that...
they would affect the results, because the basic unit of comparison in this study is IBD isoforms, which will behave consistently in any assay system (ie, the assay signal will be directly proportional to the number of Lp(a) particles for individuals expressing the same isoform).

Although we did not detect additional variance due to the method, another potential problem is in the estimation of fractional absorbance for isoforms in double-banded samples. The monoclonal antibody used in this study to estimate fractional absorbance appears to be directed against a nonrepeated epitope of apo(a).34 However, if this assumption is not true, it could affect our estimates of allele-specific concentrations. Again, larger isoforms would be estimated to occur in relatively higher and smaller isoforms in relatively lower concentrations than in reality. However, 2 factors mitigate this potential problem. First, because we included information on all family members who shared a particular IBD isoform, there was considerable variation in the identity of the second isoform band. The second isoform band could be larger or smaller, and this variation would cause the allele-specific concentration estimate for the IBD isoform to be correspondingly underestimated or overestimated, which would add “noise” to our data. Second, we are testing in this study the effects of a number of covariates on Lp(a) concentrations; there is absolutely no reason to expect that LPA genotype influences sex, age, or glucose tolerance. Thus, even if there is nonrandom error in estimating allele-specific concentrations, we would not expect this error to create an artifactual relationship with a covariate. In fact, it is likely that any analytical errors in our estimates of expected Lp(a) concentration only serve to degrade true relationships.

The main result of this study is the demonstration of a significant relationship between Lp(a) concentrations and indicators of glucose tolerance. Previously,22 we reported that diabetic subjects had significantly lower Lp(a) concentrations than nondiabetic subjects who were carefully matched for

### Table 3. Partial Correlation Coefficients (r) and P Values (Assessed by Multiple Regression Analysis) for the Relationship of Each of 4 Diabetes-Related Traits and 2 Lipid Metabolism–Related Traits With Residual Lp(a) Concentrations

<table>
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<th>Diabetes-Related Trait</th>
<th>Lipid Metabolism–Related Traits</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
</tr>
<tr>
<td>Insulin, fasting, pmol/L</td>
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</tr>
<tr>
<td>Insulin, 2 h, pmol/L</td>
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</tr>
<tr>
<td>Glucose, fasting, mmol/L</td>
<td>-0.069</td>
</tr>
<tr>
<td>Glucose, 2 h, mmol/L</td>
<td>-0.101</td>
</tr>
<tr>
<td>Nondiabetic subjects</td>
<td></td>
</tr>
<tr>
<td>Insulin, fasting, pmol/L</td>
<td>-0.105</td>
</tr>
<tr>
<td>Insulin, 2 h, pmol/L</td>
<td>-0.160</td>
</tr>
<tr>
<td>Glucose, fasting, mmol/L</td>
<td>-0.083</td>
</tr>
<tr>
<td>Glucose, 2 h, mmol/L</td>
<td>-0.054</td>
</tr>
</tbody>
</table>

Also given are multivariate R² values. Triglyceride, glucose, and insulin measures were logarithmically (e) transformed before analysis.

### Table 4. Correlation Coefficients (r) for the Relationship of 4 Measures of Glucose Metabolism With Residual Apo(a) Size as Assessed by Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
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<td></td>
<td></td>
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<tr>
<td>Insulin, fasting, pmol/L</td>
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<td>0.0013</td>
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<tr>
<td>Insulin, 2 h, pmol/L</td>
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<td>0.068</td>
<td>0.018</td>
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<tr>
<td>Glucose, fasting, mmol/L</td>
<td>1288</td>
<td>0.045</td>
<td>0.10</td>
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<tr>
<td>Glucose, 2 h, mmol/L</td>
<td>1228</td>
<td>0.064</td>
<td>0.025</td>
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<tr>
<td>Nondiabetic subjects</td>
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<tr>
<td>Insulin, fasting, pmol/L</td>
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<td>Insulin, 2 h, pmol/L</td>
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<td>0.062</td>
<td>0.045</td>
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<tr>
<td>Glucose, fasting, mmol/L</td>
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<td>0.073</td>
<td>0.016</td>
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<tr>
<td>Glucose, 2 h, mmol/L</td>
<td>1066</td>
<td>0.077</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Glucose and insulin measures were logarithmically (e) transformed before analysis.

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**Figure 4.** Frequency histograms for apo(a) size. A, Isoform sizes determined for 1288 bands in 864 samples. B, Residual apo(a) isoform sizes for the same group after subtracting IBD allele group mean size from each value.
apo(a) isoform size phenotype. The number of comparison pairs (n=81) was sufficient to detect significant effects of diabetes, but we were unable to adjust completely for genotype, as we have attempted in this study. Other studies have relied on large numbers to determine whether NIDDM was related to Lp(a) concentration. Perhaps as a result of inability to correct for the large variation due to LPA genotype, however, there has been considerable variation in conclusions, with some studies reporting higher Lp(a) levels and some lower, while most have found no significant effect.

In the current study, we found that diabetic subjects had lower residual Lp(a) concentrations than nondiabetic subjects, but this difference was not significant.

When we tested for a relationship of Lp(a) with insulin and glucose concentrations, however, there were significant correlations, especially for insulin. Lending credence to this result is the further observation that the significant correlations of insulin and Lp(a) exist even in a subset of nondiabetic individuals (Table 2). The general trend of NIDDM is for Lp(a) concentrations that are lower than expected for an individual’s genotype. Therefore, Lp(a) cannot help explain the “surplus” of cardiovascular disease found in NIDDM patients.

Previously, we demonstrated significant correlations of Lp(a) with 2 lipid measures, plasma concentrations of cholesterol and triglycerides. We tested whether the relationship of Lp(a) with insulin and glucose might be mediated by general effects on lipoprotein metabolism (ie, whether inclusion of lipid measures would abolish the significant relationships). Multiple regression analyses, however, demonstrated that although the 2 lipid variables were strongly related to residual Lp(a), the relationships with insulin and glucose remained significant and thus, were independent.

The data do not suggest a mechanism responsible for the relationship between Lp(a) and indicators of diabetes status. NIDDM is characterized by hyperinsulinemia, and we speculate that variation in insulin concentrations regulates apo(a) biosynthesis, even in nondiabetic subjects. This speculation is founded on several recent studies of cultured hepatocytes. Insulin concentrations inhibit, in a dose-dependent manner, Lp(a) production by cyanomolgous primary hepatocytes and expression of an apo(a) S’-flanking region-reporter construct in HepG2 cells. Because IDDM is not characterized by hyperinsulinemia, it seems likely that other factors, perhaps at the catabolic level, are responsible for the observed increases of Lp(a) concentrations in these patients.

A second finding of this study is that apo(a) isoforms were significantly larger in individuals who had elevated insulin or glucose levels. We presume that this is due to the general process of nonenzymatic glycation of plasma proteins in hyperglycemia. This result confirms our earlier study with far fewer isoforms in the comparison and also the study of Doucet et al.

In this report we have shown a small, but consistent, association of insulin and Lp(a) levels. Other reports in this area have been controversial, perhaps because of failure to account for strong genetic effects on Lp(a) concentrations. Previously, we have shown that Lp(a) particle concentration and composition are both significantly related to triglyceride concentrations, which are elevated in insulin resistance. Furthermore, we have shown that Lp(a) density (size) is significantly related to particle sizes of both HDL and LDL, which are reduced in response to insulin resistance. Together with the present results demonstrating significant relationships of Lp(a) concentration and apo(a) size with measures of insulin and glucose, it is apparent that Lp(a) is another, among many lipoproteins, that is associated with the metabolic syndrome characterized by insulin resistance. This conclusion needs to be tested further in studies of pharmacological agents that improve insulin sensitivity.

Acknowledgments

This work was supported in part by NIH grants HL45522 and HL50521 (D.L.R.). The authors are grateful for technical help from Janie Ludwig and Rosa Roselló and the kind gift of the monoclonal antibody 2D1.

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\textit{Arterioscler Thromb Vasc Biol.} 1998;18:1335-1341
doi: 10.1161/01.ATV.18.8.1335

\textit{Arteriosclerosis, Thrombosis, and Vascular Biology} is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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