Lack of Association Between Sex Hormones and Lp(a) Concentrations in American and Finnish Men

Steven M. Haffner, Leena Mykkanen, Katherine K. Gruber, David L. Rainwater, Markku Laakso

Abstract Sex hormones may play a role in the determination of cardiovascular disease. Recently lipoprotein(a) (Lp(a)) has been recognized as a risk factor for coronary heart disease. Estrogens and anabolic steroids have been reported to alter Lp(a) levels, yet no data are available on the association between in vivo concentrations of sex hormones and Lp(a) concentrations. We examined the possible associations of sex hormone–binding globulin, total and free testosterone, estradiol, and dehydroepiandrosterone sulfate to Lp(a) concentrations in men in two population-based studies (San Antonio Heart Study [n=178] and a Finnish study on the association between insulin resistance and atherosclerosis [n=87]). In neither study were sex hormones significantly related to Lp(a) concentrations. In addition, Lp(a) was significantly related to apolipoprotein(a) molecular weight (which was measured in the Finnish study only). These results were unchanged when Lp(a) concentrations were adjusted for apolipoprotein(a) molecular weight (a strong correlate of Lp(a) concentrations). We conclude that in vivo concentrations of sex hormones are unlikely to be associated with Lp(a) concentrations in men.

Key Words • sex hormones • lipoprotein(a)

Sex hormones may play a role in the determination of risk of cardiovascular disease. Indirect evidence for this concept is provided by the higher triglyceride and low-density lipoprotein cholesterol (LDL-C) and lower high-density lipoprotein cholesterol (HDL-C) levels in men than women.1 In vivo, sex hormones have been related to cardiovascular risk factors in men and women.2,10 Interestingly, the association of testosterone with lipids and lipoproteins may be different in men than women. Increased levels of free testosterone and decreased levels of sex hormone–binding globulin (SHBG) are associated with atherogenic changes in lipid and lipoprotein profiles (especially increased triglyceride and decreased HDL-C concentrations) in premenopausal and postmenopausal women.5–7 However, increased testosterone concentrations in men have been associated with higher HDL-C levels in most8 but not all9,10 studies.

Recently, lipoprotein(a) (Lp(a)) has been associated with increased coronary heart disease.11–15 The determinants of Lp(a) concentration have a major genetic component.16,17 Since Lp(a) concentrations do not vary between men and women,16–20 it would be expected that sex hormones would not have a major effect on Lp(a) concentration. However, a number of observations do suggest a possible role for sex hormones in modulating Lp(a) concentrations. Lp(a) levels are higher in postmenopausal than premenopausal women.19,21 Postmenopausal estrogen accompanied by progestagen therapy has been reported to lower Lp(a) concentrations.22,23 Pharmacological intervention with anabolic steroids in men and women24 and estrogen therapy in men with prostatic carcinoma25 have been shown to reduce Lp(a) concentrations. Orchiectomy in men with prostatic cancer has led to increases in Lp(a) concentrations.25 Lp(a) levels have been found to be higher in postpubertal than prepubertal subjects with insulin-dependent diabetes mellitus.26,27

To date, no study has examined the association of in vivo sex hormones and binding proteins with Lp(a) concentrations. In this report, we examine the influence of total and free testosterone, dehydroepiandrosterone sulfate, estradiol, and SHBG on plasma Lp(a) concentrations in men from two population-based studies in the United States and Finland. Since obesity, body fat distribution, and hyperinsulinemia may be associated with alterations in sex hormones,2,5–7 we have controlled for these factors. In addition, because genetic factors may have a major influence on Lp(a) concentration,16,17 we have also accounted for apolipoprotein(a) (apo[a]) phenotype effects on Lp(a) concentrations in the Finnish population.

Methods

San Antonio Heart Study

The San Antonio Heart Study is a population-based study of diabetes and cardiovascular disease in Mexican Americans and non-Hispanic whites. Between 1979 and 1982, 1288 Mexican Americans and 929 non-Hispanic white men and nonpregnant women, aged 25 to 64 years old when first examined, were randomly selected from three neighborhoods in San Antonio, Tex. Mexican Americans are defined as individuals whose ancestry and cultural traditions are derived from a Mexican national origin.28 The response rate was 63.9%. A detailed description of the survey has been published.29 The study was approved by the Institutional Review Board of the University of Texas Health Science Center, 7703 Floyd Curl Dr, San Antonio, TX 78284-4737.
of Texas Health Science Center at San Antonio, and all subjects gave informed consent. In October 1987, follow-up of the 1979 to 1982 cohort was begun to ascertain the incidence of non-insulin-dependent diabetes mellitus, hypertension, and coronary heart disease. Vital status was ascertained for 97.8% of the originally enrolled subjects. The follow-up examination consisted of an initial home interview (completed by 96.9% of the surviving subjects) followed by a medical examination (which was attended by 82.9% of the subjects who completed the telephone interview). The overall response rate was 80.3% (0.969 x 0.829). A total of 969 Mexican Americans and 704 non-Hispanic whites attended the follow-up examination. Details of the follow-up study have been published. The analyses in this report are based on 178 male subjects for whom fasting serum samples were available and who lived in a middle-income neighborhood (sixth census tracts of the follow-up study).

Anthropometric measurements (height, weight, and waist and hip circumferences) were done with the participant's wearing an examination gown after removal of his shoes and upper garments. Waist circumference was measured at the level of the umbilicus and hip circumference at the level of the greater trochanter. The average of two readings was used as the measurement for each circumference. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared, and BMI was used as an index of overall adiposity. The ratio of waist-to-hip circumference (WHR) was used as a measure of upper body adiposity.

Blood samples were obtained after a 12-hour fast. Methods for determination of serum lipids and lipoproteins have been described. Glucose levels were measured by a glucose oxidase method, and insulin was measured with a commercial solid-phase radioimmunoassay (Diagnostic Products Corp, Los Angeles, Calif). After the fasting blood samples were obtained, a 75-g glucoseload was administered orally. Blood samples for plasma glucose and plasma insulin concentrations were obtained 1 and 2 hours after the glucose load. Blood glucose in the fasting state was measured by the glucose oxidase method. Plasma insulin was determined by radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). Serum lipid and lipoprotein levels were determined from fresh serum samples drawn after a 12-hour overnight fast. Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation as described. Cholesterol and triglyceride levels from whole sera and from lipoprotein fractions were assessed by automatic enzymatic methods (Boehringer-Mannheim, Mannheim, FRG). Sex hormones and Lp(a) were measured in sera (frozen at —70°C for an average of 25 months) in the laboratory of the San Antonio Heart Study using methods that were identical to those used in the San Antonio Heart Study.

**Determination of Apo(a) Molecular Weight**

Apo(a) isoform sizes were determined by immunochromatography. From a separate serum aliquot that was frozen at —70°C for an average of 12 months before the assay, Lp(a) was measured by a monoclonal Lp(a) antibody technique (Terumo Medical Corp, Elkton, Md). The intra-assay and interassay coefficients of variation for Lp(a) were 4% and 8%, respectively. In a recent report of this monoclonal antibody technique, no cross-reactivity with plasminogen, LDL-C, very-low-density lipoprotein cholesterol, or HDL-C was observed. Apo(a) isoforms were also phenotyped into five size categories, with A having the largest molecular weights and E the smallest. The estimated average molecular weights of isoform categories for this study were 78 ± 0.00, 695 ± 0.00, and 980 000 for A, B, and C, respectively; the other two isoform categories (D and E) were observed in only four individuals. There was one null-phenotype individual in the study, which indicated that neither allelic product was detected in the serum sample.

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An ANOVA (Table 4) was performed with only the common
The following statistical tests (SAS)\(^4\) were used: \(t\) tests (Table 1), Pearson correlation coefficients (Tables 2 and 3), ANOVA (Table 4), and partial correlation analysis (Tables 2 and 3). Log transformations of Lp(a) and triglyceride concentrations were used to improve adherence to an assumed gaussian distribution for use with \(t\) tests (Table 1), Pearson and partial correlations (Tables 2 and 3), and ANOVA (Table 4). The analyses in this report were initially performed separately for Mexican Americans and non-Hispanic whites. Since relations between sex hormones and Lp(a) were similar in Mexican Americans and non-Hispanic whites (ie, slope of the correlation coefficient) (Table 2), the two phenotypes (ie, three or more individuals with that phenotype).

### Statistical Methods

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### Table 1. Means and Standard Errors for Clinical Characteristics, Lipids, Lipoproteins, Lp(a), Sex Hormones, and Apo(a) Molecular Weight

<table>
<thead>
<tr>
<th>San Antonio Heart Study Population</th>
<th>Mexican Americans</th>
<th>Non-Hispanic Whites</th>
<th>(P^*)</th>
<th>Finnish Insulin Resistance and Atherosclerosis Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>75</td>
<td>103</td>
<td>87</td>
<td>[681.2±0.8]</td>
</tr>
<tr>
<td>Age, y</td>
<td>53.0±1.2</td>
<td>52.1±1.2</td>
<td>.575</td>
<td>54.2±0.6</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>27.6±0.5</td>
<td>27.1±0.5</td>
<td>.223</td>
<td>26.3±0.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.969±0.006</td>
<td>0.966±0.003</td>
<td>.631</td>
<td>0.968±0.006</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>26.1±2.2</td>
<td>29.4±2.1</td>
<td>.346</td>
<td>34.0±0.5</td>
</tr>
<tr>
<td>Free testosterone, pg/mL</td>
<td>21.8±0.8</td>
<td>21.6±0.7</td>
<td>.444</td>
<td>17.1±0.1</td>
</tr>
<tr>
<td>Total testosterone, ng/mL</td>
<td>6.63±0.41</td>
<td>6.27±15.5</td>
<td>.445</td>
<td>5.56±0.03</td>
</tr>
<tr>
<td>DHEA-SO(_4), mg/dL</td>
<td>241.4±16.6</td>
<td>242.7±15.5</td>
<td>.903</td>
<td>176.6±0.8</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>54.7±1.6</td>
<td>52.3±1.6</td>
<td>.985</td>
<td>37.4±0.13</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>2.12±0.24</td>
<td>2.08±0.23</td>
<td>.908</td>
<td>1.49±0.11</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.99±0.03</td>
<td>1.03±0.03</td>
<td>.630</td>
<td>1.28±0.03</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.49±0.13</td>
<td>5.45±0.11</td>
<td>.891</td>
<td>5.96±0.12</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.64±0.23</td>
<td>3.85±0.10</td>
<td>.801</td>
<td>3.99±0.11</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>11.8±1.5</td>
<td>16.4±1.7</td>
<td>.251</td>
<td>16.5±0.2</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.5±0.3</td>
<td>5.3±0.2</td>
<td>.180</td>
<td>5.6±0.1</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>115.1±19.4</td>
<td>89.2±6.5</td>
<td>.736</td>
<td>66.9±4.3</td>
</tr>
<tr>
<td>Molecular weight of apo(a), (\times1000)</td>
<td>...</td>
<td>...</td>
<td>681.2±0.8</td>
<td></td>
</tr>
</tbody>
</table>

\(Lp(a)\) indicates lipoprotein(a); Apo(a), apolipoprotein(a); BMI, body mass index; WHR, waist-to-hip ratio; SHBG, sex hormone-binding globulin; DHEA-SO\(_4\), dehydroepiandrosterone sulfate; HDL, high-density lipoprotein; and LDL, low-density lipoprotein. Probability values were derived from the \(t\) test.

*Probability values compare Mexican Americans with non-Hispanic whites.

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### Table 2. Correlations of Lp(a) With Sex Hormones in Men in the San Antonio Heart Study

<table>
<thead>
<tr>
<th>Univariate</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>MAs</td>
</tr>
<tr>
<td>SHBG</td>
<td>.128</td>
</tr>
<tr>
<td></td>
<td>(.294)</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>.097</td>
</tr>
<tr>
<td></td>
<td>(.428)</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>.122</td>
</tr>
<tr>
<td></td>
<td>(.320)</td>
</tr>
<tr>
<td>DHEA-SO(_4)</td>
<td>-.030</td>
</tr>
<tr>
<td></td>
<td>(.807)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>.025</td>
</tr>
<tr>
<td></td>
<td>(.841)</td>
</tr>
</tbody>
</table>

\(Lp(a)\) indicates lipoprotein(a); MAs, Mexican-Americans; NHWs, non-Hispanic whites; SHBG, sex hormone-binding globulin; and DHEA-SO\(_4\), dehydroepiandrosterone sulfate. Univariate correlations are parametric Pearson correlations and partial correlations are parametric correlations. Probability values are shown in parentheses.

*Adjusted for age, ethnicity, body mass index, waist-to-hip ratio, fasting glucose, and fasting insulin.
DHEA-SO₄, dehydroepiandrosterone sulfate. Univariate correlations are parametric Pearson correlations and multivariate correlations are parametric correlations. Probability values are shown in parentheses.

Table 3 shows correlations between sex hormones, Lp(a) concentrations, and apo(a) molecular weights in the Finnish study. Neither Lp(a) concentration nor apo(a) size was significantly related to any of the sex hormones. Lp(a) concentration was significantly related to apo(a) molecular weight (r = -0.600, P < 0.001). After adjustment for age, BMI, WHR, fasting glucose, and fasting insulin, Lp(a) concentrations were not significantly related to any of the sex hormones (data not shown).

Table 4 shows apo(a) molecular weights and concentrations of sex hormones and Lp(a) by apo(a) letter phenotype in the Finnish study. Molecular weights of apo(a) and Lp(a) concentrations were significantly related to apo(a) letter phenotype (P < 0.001). However, none of the sex hormones was significantly related to apo(a) phenotype.

**Discussion**

We have shown in this report that sex hormone concentrations are not related to Lp(a) concentrations in two population-based studies. Since the lack of association between Lp(a) concentrations and sex hormones occurs in Caucasian populations in different parts of the world as well as in Mexican Americans (a population with increased Native American admixture), these results are likely to be generalizable. In addition, the number of subjects in these samples was large. The lack of association between Lp(a) and sex hormones is not affected by adjustment for a number of factors that are known to affect concentrations of sex hormones, such as obesity, body fat distribution, and insulin and glucose concentrations.

Genetic factors are a major determinant of Lp(a) concentration. The apo(a) gene (LPA gene) may account for >90% of the variation in Lp(a) concentration. Thus, apo(a) phenotype may have major influences on the association of environmental factors (such as sex hormones) with Lp(a) concentration. As observed by others, there was a strong inverse correlation between apo(a) molecular weight and Lp(a) concentration in this study. However, adjusting Lp(a) concentration for apo(a) phenotype did not alter the lack of association between Lp(a) concentrations and sex hor-

### Table 4. Concentrations of Lp(a) and Sex Hormones by Apo(a) Phenotype in Finnish Men (n=82)

<table>
<thead>
<tr>
<th>Apo(a) Phenotype</th>
<th>A*</th>
<th>B</th>
<th>AB</th>
<th>AC</th>
<th>BC</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>23</td>
<td>14</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>5.1</td>
<td>14.1</td>
<td>16.8</td>
<td>33.6</td>
<td>34.8</td>
<td>25.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Apo(a) molecular weight, x1000</td>
<td>788</td>
<td>693</td>
<td>690</td>
<td>594</td>
<td>598</td>
<td>602</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>31.2</td>
<td>29.6</td>
<td>29.6</td>
<td>28.2</td>
<td>34.5</td>
<td>27.2</td>
<td>.286</td>
</tr>
<tr>
<td>Total testosterone, ng/mL</td>
<td>5.3</td>
<td>5.7</td>
<td>5.9</td>
<td>4.8</td>
<td>6.4</td>
<td>5.2</td>
<td>.773</td>
</tr>
<tr>
<td>Free testosterone, pg/mL</td>
<td>17.2</td>
<td>17.5</td>
<td>17.8</td>
<td>14.2</td>
<td>19.2</td>
<td>15.8</td>
<td>.587</td>
</tr>
<tr>
<td>DHEA-SO₄, mg/dL</td>
<td>180</td>
<td>160</td>
<td>186</td>
<td>190</td>
<td>223</td>
<td>156</td>
<td>.301</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>36.2</td>
<td>37.1</td>
<td>40.2</td>
<td>37.2</td>
<td>41.3</td>
<td>36.2</td>
<td>.723</td>
</tr>
</tbody>
</table>

Lp(a) indicates lipoprotein(a); Apo(a), apolipoprotein(a); SHBG, sex hormone-binding globulin; and DHEA-SO₄, dehydroepiandrosterone sulfate. One subject had BE, 2 subjects had AE, 1 subject had AD, and 1 subject had a null phenotype. These subjects were excluded from this analysis. Probability values were calculated by ANOVA.

*Three subjects had an AA pattern (pseudohomozygosity).
mones. Of course, apo(a) molecular weight alone does not explain all genetic variation in Lp(a) concentration. It is possible that correcting for other factors that control Lp(a) concentration will reveal a relation between Lp(a) concentrations and sex hormones.

The lack of association between in vivo sex hormones and Lp(a) concentrations contrasts with the effects of estrogen or anabolic steroid treatment on Lp(a) concentration. The observation that both exogenous estrogen and anabolic steroids decrease Lp(a) levels suggests that the effects of these hormones are related to initial effects on liver metabolism. The major source of Lp(a) variation in normal subjects is due to differences in Lp(a) synthesis in the liver. Differences in the effect of exogenous testosterone and anabolic steroid administration on HDL concentrations have also been reported. Anabolic steroids have been associated with decreased HDL-C and increased LDL-C concentrations. However, these differences may be partially due to different routes of administration, since anabolic steroids are usually given orally and testosterone is usually administered by intramuscular injection. One explanation for the differences between oral androgens and intramuscular testosterone on HDL-C levels is that testosterone readily aromatizes to 17β-estradiol, whereas oral androgens do not form potent estrogens. Administration of testosterone enanthate with testosterone (which inhibits aromatization of testosterone to 17β-estradiol) leads to increases in hepatic triglyceride lipase and decreases in HDL-C. The latter observation suggests that aromatization to estradiol is responsible for the testosterone effect, which does not occur with anabolic steroid use. An intramuscular testosterone model may thus provide a better model for the effect of in vivo sex hormones on Lp(a) concentrations.

In conclusion, we have demonstrated a lack of effect of sex hormones on Lp(a) concentrations in men. These results were not affected by adjustment for confounding variables, such as obesity and insulin concentrations, nor were they affected by adjustment for apo(a) molecular weight. These studies should be repeated in women. The paradox between the lowering of Lp(a) concentrations by estrogen or anabolic steroid treatment and the current findings that show no association of in vivo sex hormones with Lp(a) concentrations might be partially resolved by examining the effect of intramuscular testosterone. Perhaps intramuscular testosterone does not affect Lp(a) concentrations. Measurement of apo(a) phenotype would also be useful, since the effect of sex hormones might differ by phenotype.

Acknowledgments

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References


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