Significant Association Between Low-Molecular-Weight Apolipoprotein(a) Isoforms and Intermittent Claudication

Jørgen Mølgaard, Ib Christian Klausen, Claes Lassvik, Ole Færgeman, Lars Ulrik Gerdes, and Anders G. Olsson

The role of lipoprotein(a) (Lp[a]) and apolipoprotein(a) (apo[a]) isoforms in symptomatic peripheral atherosclerosis was studied in 100 randomly selected middle-aged (45-69 years) men with intermittent claudication (IC) and 100 randomly selected healthy control (C) subjects. IC and C subjects were matched pairwise for sex, age, and smoking habits. Plasma Lp(a) concentrations were significantly higher in IC subjects, with a median value of 20.12 mg/dl, compared with 11.11 mg/dl in C subjects (p<0.0009). The elevated Lp(a) concentration was to a great extent due to a significant difference in the frequency distribution of apo(a) isoforms between IC and C subjects (p<0.029). Low-molecular-weight apo(a) isoforms were more prevalent in IC than C subjects. Also, IC subjects with apo(a) S2 and S3 phenotypes had higher Lp(a) concentrations than control subjects with the same phenotypes: S2: 60.70 mg/dl (IC) and 48.69 mg/dl (C), p<0.038; and S3: 30.18 mg/dl (IC) and 12.01 mg/dl (C), p<0.042, so other still-unknown factors, genetic or nongenetic, may be important. Stepwise logistic regression analysis demonstrated that Lp(a) concentration contributed significantly (p<0.0002) to IC, independent of age, smoking, hypertension, diabetes mellitus, plasma total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, apo B, and plasma total triglycerides. Apo(a) isoforms grouped according to molecular weight were also independent of the above risk factors associated (p=0.016) with the occurrence of IC because of their low-molecular-weight but were not independent of Lp(a) concentrations. Because IC and C subjects had similar smoking habits, the study also suggests that elevated Lp(a) levels due to a preponderance of low-molecular-weight apo(a) isoforms may be an explanation for the difference in risk that smokers have for developing IC.

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KEY WORDS • lipoprotein(a) • apolipoprotein(a) isoforms • intermittent claudication • risk factors • epidemiology

Peripheral atherosclerosis is a multifactorial disease. The majority of patients with intermittent claudication (IC) have a positive history of smoking. Other major risk factors are diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, and low levels of high density lipoprotein (HDL) cholesterol.

Another important lipid factor associated with atherosclerosis is lipoprotein(a) (Lp[a]), which was discovered in 1963 by Käre Berg. Lp(a) is composed of a low density lipoprotein (LDL)-like lipoprotein and a glycoprotein called apolipoprotein(a) (apo[a]). The LDL moiety and apo(a) are thought to be connected by a disulfide bridge between the apo B of LDL and apo(a). In whites the plasma Lp(a) concentration is to a large extent genetically determined, and one major multiallelic locus controls the trait. The frequency distribution of Lp(a) concentrations is continuous and highly left-skewed in white populations but is bell-shaped in black populations. The apo(a) gene locus determines a number of isoforms of apo(a), with apparent molecular weights ranging from approximately 400 to 800 kd. The apo(a) isoforms are usually grouped into six major categories: F, B, S1, S2, S3, S4, and a "null group," a classification originally described by Utermann et al. The Lp(a) concentrations vary inversely with the molecular weight of the apo(a) isoforms. About 40% of the total variability in Lp(a) plasma concentrations in whites is determined by the apo(a) locus.

Elevated Lp(a) concentrations are associated with coronary heart disease (CHD). The serum Lp(a) concentration is also a predictor of vein graft occlusion after coronary artery bypass surgery and of CHD in subjects with familial hypercholesterolemia. An association between elevated Lp(a) level and stroke has also been shown.

The present study investigated the following two questions. 1) Is elevated Lp(a) concentration associated with IC? 2) Is it independent of other classical risk factors?
factors for peripheral atherosclerosis, such as smoking, diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, and low HDL cholesterol concentrations? To avoid the possible influence of age and sex as well as selection bias, both IC and control subjects were randomly selected and matched for sex and age. Because asymptomatic peripheral artery disease is not uncommon, control subjects were also subjected to a treadmill exercise test to exclude those with hemodynamically significant peripheral artery disease.

**Methods**

**Subjects**

The study population was recruited from an epidemiological study of IC in Linköping County, Sweden, where all middle-aged men (45–69 years old, n=15,253) were screened for symptoms suggesting IC by a Rose questionnaire that was sent by mail. The questionnaire also contained detailed questions about smoking habits. Eighty-seven percent (n=13,203) responded to the questionnaire, and 1.2% had symptoms indicating IC. From this population, 103 subjects with verified IC and 103 healthy control subjects matched for sex, age (±2 years), and smoking habits were randomly selected. IC and control subjects were matched pairwise for smoking habits. Both IC and control subjects underwent a physical examination by the same physician, and a detailed history was taken concerning IC and other signs or manifestations of clinical atherosclerosis, such as angina pectoris, myocardial infarction, transient ischemic attacks (TIAs), and stroke. Hospital records were checked to verify such events. Of 54 patients with CHD, 32 had experienced myocardial infarction and 17 had received coronary artery bypass grafts. Thirteen patients had experienced a stroke or a TIA.

The percentages of subjects with a smoking history, hypertension, and diabetes mellitus were 97%, 47%, and 14% in the IC group and 97%, 7%, and 0% in the control group, respectively. The diagnosis of IC was based on a combination of 1) typical symptoms of pain in the legs when walking at a fast pace or uphill that was relieved within 10 minutes by slowing down or resting and 2) an abnormal treadmill exercise test as described later. The control subjects were all healthy without symptoms of IC.

About half of the patients (n=43) had previously undergone distal aortofemoral angiography or ultrasound examination (n=2) showing typical atherosclerotic changes with stenosis and/or occlusions. Forty-eight of the patients had previously had an abnormal standard treadmill exercise test. Thirty-two patients had undergone operation for IC. Irrespective of previous diagnostic procedures, all subjects with IC symptoms and all control subjects underwent a standardized treadmill exercise test.

**Blood Sampling**

Venous blood samples were taken after at least 12 hours of fasting for analysis of plasma Lp(a) concentration, apo(a) isoforms, cholesterol and triglyceride concentrations in plasma and major lipoprotein fractions, apo A-I and B concentrations, hematology and blood chemistry parameters, liver function tests, and blood glucose and thyroid function tests.

**Determination of Lp(a) Concentrations**

Samples for determination of plasma lipoprotein concentrations were collected in vacuum tubes containing NaEDTA. The tubes were placed on ice and centrifuged, and plasma was immediately stored at −70°C until analysis. Samples were shipped in dry ice to Århus, Denmark.

Samples from case and control subjects were processed at the same time and with the same batch of reagents. Lp(a) was measured with a commercial two-site immunoradiometric assay (Pharmacia Diagnostics AB, Uppsala, Sweden). The method employs two different monoclonal apo(a) antibodies, one labeled with 125I and one coupled to micro-Sepharose. The standards and samples were incubated with an excess of both antibodies, and the resulting antigen–antibody complexes were separated from unbound 125I by centrifugation. The bound iodine was measured in a gamma counter. A standard curve was constructed for each run, and samples were analyzed in duplicate in the same run. The intra-assay coefficient of variation was 2.2%, and the interassay coefficient of variation was 3.1%. The lower limit of detection in the assay was 1.68 mg/dl. The Århus laboratory participates in an international Lp(a) standardization program. Jauhiainen et al. have demonstrated that storage of serum even at −20°C for 7 years does not affect Lp(a) concentrations measured by the method we used.

**Determination of Apo(a) Phenotypes**

Apo(a) isoforms (Figure 1) were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting by a method slightly modified from that described by Utermann et al. The samples for analyses were prepared by mixing 4 μl plasma (null phenotypes were repeated with 6–8 μl) with 50 μl of a buffer prepared from 5% SDS (BHD Chemicals, UK) and 0.02 M ethylmorpholine. Four microliters of β-mercaptoethanol (Merck, Darmstadt, FRG) and 2 μl of 1.5% bromophenol blue in glycerol were added, and the samples were placed in a boiling-water bath for 10 minutes. Aliquots of 25 μl were then subjected to SDS-PAGE in a discontinuous buffer and gel system with a stacking gel solution of 3.6% polyacrylamide and a resolving gel solution of 6.8% polyacrylamide. The upper 2.5 cm of the glass plates (stacking gel area) were treated with bind-silane (A-174, LKB, Bromma, Sweden). Acrylamide and N,N'-methylen-bis-acrylamide were purchased from LKB, and the electrophoresis equipment was of the Desaphor va. type from Desaga. Proteins were transferred to nitrocellulose filters (BA85, 0.45 μm, Schle-
was repeated, with measurements taken on the other
pressures were measured immediately, after 1.5 minutes,
2 minutes, and every minute for 5 minutes after the
exercise. After 10 minutes of rest the exercise test
was repeated, with measurements taken on the other
leg.36 A resting systolic ankle to arm pressure index
\( \leq 0.90 \) or a fall in systolic ankle pressure of \( \geq 30 \) mm Hg
immediately after exercise was considered abnormal.37

**Resting Blood Pressure**

Systolic and diastolic blood pressures were measured
in both arms by auscultation by using a mercury ma-
nometer and a 12-cm cuff after the subjects had rested
for at least 10 minutes.

**Statistics**

Nonparametric tests were used because of the highly
skewed frequency distribution of Lp(a) concentrations.
The Mann-Whitney \( U \) test was used for assessing statis-
tical differences in variables between the IC and control
groups. The Kruskal-Wallis and \( y^2 \) tests were used to
assess differences in Lp(a) concentrations according to
apo(a) phenotypes and frequency distributions. The
Spearman rank correlation was performed to test associ-
ations of Lp(a) with other variables.

To determine the role of plasma Lp(a) in IC in relation
to known risk factors, stepwise logistic regression
analysis was performed with the disease as the depen-
dent variable and then sequentially adding age, smok-
ing, hypertension, diabetes mellitus, total plasma
cholesterol, total triglycerides, HDL cholesterol, and
plasma Lp(a) as the last entry. For significance testing,
log likelihood ratio statistics were used.

The study protocol was approved by the Ethics Com-
mittee of the Faculty of Health Sciences, University
Hospital, Linköping.

**Results**

Three control subjects were excluded because of a
pathological treadmill test; also excluded were their
pairwise-matched IC subjects.

The median Lp(a) concentration was significantly
higher in IC subjects (20.12 mg/dl) than in control
subjects (11.11 mg/dl; \( p < 0.001 \); Table 1). Both fre-
cuency distributions of Lp(a) were skewed toward low
concentrations of Lp(a), but this trend was less pro-
nounced in IC than in control subjects (Table 1 and
Figure 2). The frequency distribution of apo(a) pheno-
types also differed significantly between the groups
(likelihood ratio, \( p < 0.029 \)). IC subjects had a higher
frequency of the low-molecular-weight apo(a) isoform
S2 (25% in IC versus 11% in control subjects) and a
lower frequency of the high-molecular-weight isoform
S4 (24% in IC versus 40% in control subjects) (Table 1).
Lp(a) concentrations were significantly associated with
the apo(a) phenotypes (Kruskal-Wallis test, \( p < 0.0001 \)).
As expected, the Lp(a) concentrations were highest in
individuals with the low-molecular-weight phenotypes
(Table 1). There was also a general tendency for IC
subjects to have higher Lp(a) concentrations than con-
trol subjects with the same apo(a) phenotype. For
apo(a) phenotypes S2 and S3, these differences were
significant: for S2, 60.70 mg/dl in IC versus 48.99 mg/dl
in control subjects, \( p < 0.038 \); for S3, 30.18 mg/dl in IC
versus 12.01 mg/dl in control subjects, \( p < 0.042 \) (Table
1). The frequency of single-band apo(a) phenotypes did
not differ between the groups; 67% in the IC and 72% in
the control group.

Conventional lipid risk factors also differed between
IC and control subjects. Total, VLDL, and LDL chole-
sterol concentrations were significantly higher and HDL...
cholesterol levels significantly lower in IC compared with control subjects (Table 2). The most pronounced difference in the lipid parameters was found in total and VLDL triglycerides. The apo B concentration was also significantly higher in IC patients, whereas apo A-I was significantly lower (Table 2).

Various clinical and laboratory parameters are shown in Table 3. Body mass index did not differ significantly between the groups, whereas plasma fibrinogen level, sedimentation rate, leukocyte count, and serum creatinine level were moderately increased in IC subjects.

Spearman rank correlations between Lp(a) concentration and various parameters showed a minor but significant correlation with apo B in both groups (r=0.20, p<0.002 in the IC and r=0.24, p<0.008 in the control group). In IC subjects but not in control sub-

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### TABLE 1. Plasma Lp(a) Concentrations for Each Apo(a) Phenotype in Subjects With Intermittent Claudication and Healthy Control Subjects Matched for Sex, Age, and Smoking Habits

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>IC subjects</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lp(a) conc</td>
<td>n</td>
</tr>
<tr>
<td>All subjects</td>
<td>20.12 (1.68–322.20)</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>101.34</td>
<td>1</td>
</tr>
<tr>
<td>BS4</td>
<td>76.30 (46.61–121.46)</td>
<td>10</td>
</tr>
<tr>
<td>S1</td>
<td>322.20</td>
<td>1</td>
</tr>
<tr>
<td>S1S2</td>
<td>124.24</td>
<td>1</td>
</tr>
<tr>
<td>S1S3</td>
<td>60.70 (4.07–205.96)</td>
<td>25</td>
</tr>
<tr>
<td>S1S4</td>
<td>77.97 (54.29–101.64)</td>
<td>2</td>
</tr>
<tr>
<td>S2</td>
<td>19.39</td>
<td>7</td>
</tr>
<tr>
<td>S2S3</td>
<td>30.18</td>
<td>13</td>
</tr>
<tr>
<td>S2S4</td>
<td>11.79 (1.68–29.58)</td>
<td>10</td>
</tr>
<tr>
<td>S3</td>
<td>12.41 (3.20–36.33)</td>
<td>24</td>
</tr>
<tr>
<td>S4</td>
<td>1.68 (1.68–7.32)</td>
<td>11</td>
</tr>
</tbody>
</table>

Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); IC, intermittent claudicants; conc, concentration; ND, no significance testing done because of small numbers; NS, not significant. Lp(a) values are in milligrams per deciliter and are the median and (range).

Pairwise comparison of IC and control subjects for each phenotype; p=two-tailed significance values by Mann-Whitney U test.
TABLE 2. Plasma Lipids, Lipoproteins, and Apolipoproteins A-I and B in Patients With Intermittent Claudication and Healthy Control Subjects Matched for Age, Sex, and Smoking Habits

<table>
<thead>
<tr>
<th>Lipid variables</th>
<th>IC subjects (n = 100)</th>
<th>Control subjects (n = 100)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol*</td>
<td>5.63±1.05</td>
<td>5.26±0.87</td>
<td>&lt;0.011</td>
</tr>
<tr>
<td>Total triglycerides*</td>
<td>1.72±1.15</td>
<td>1.11±0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL cholesterol*</td>
<td>0.56±0.55</td>
<td>0.32±0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL triglycerides*</td>
<td>1.25±1.08</td>
<td>0.73±0.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol*</td>
<td>4.06±0.96</td>
<td>3.73±0.81</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>HDL cholesterol*</td>
<td>0.95±0.29</td>
<td>1.17±0.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>1.23±0.19</td>
<td>1.31±0.15</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Apolipoprotein B*</td>
<td>1.39±0.30</td>
<td>1.25±0.29</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. Values are mean±SD and are in millimoles per liter* or grams per liter*.

p=two-tailed significance values by Mann-Whitney U test.

..., plasma fibrinogen, LDL cholesterol, total plasma cholesterol, and sedimentation rate were weakly correlated with Lp(a) (Table 4). Logistic regression analysis with IC as the dependent variable and subsequent stepwise addition of smoking, hypertension, diabetes mellitus, total cholesterol, HDL cholesterol, total triglyceride, and Lp(a) concentration as the last entry showed that Lp(a) contributed significantly to the model (p<0.0002). Using LDL cholesterol as apo(a) type instead of total cholesterol in the model yielded similar results. Apo(a) isoforms can be grouped into three types: those of high molecular weight (S3, S3S4, and S4), phenotype "0" (null phenotype), and low molecular weight (presence of F, B, S1, or S2). Using apo(a) types instead of Lp(a) concentration in the model yielded similar results. Apo(a) isoforms: those of high molecular weight (S3, S3S4, and S4), phenotype "0" (null phenotype), and low molecular weight (presence of F, B, S1, or S2). Using apo(a) types instead of Lp(a) concentration in the model yielded similar results. Apo(a) isoforms: those of high molecular weight (S3, S3S4, and S4), phenotype "0" (null phenotype), and low molecular weight (presence of F, B, S1, or S2).

The present study shows a significant elevation of Lp(a) in patients with IC compared with healthy control subjects matched for sex, age, and smoking habits. The elevation of Lp(a) concentrations is mainly due to differences in the frequency distribution of apo(a) isoforms, with a preponderance of low-molecular-weight isoforms in IC subjects. Apo(a) isoforms are determined by one multiallelic gene locus on chromosome 6p that determines about 40% of the variation in the concentration of Lp(a) in plasma in whites. The study thus suggests that apo(a) polymorphism could be an important genetic risk factor for IC.

In a recently published study, it was observed that patients with familial hypercholesterolemia and CHD had a significantly higher frequency of lower-molecular-weight apo(a) isoforms and higher Lp(a) plasma concentrations than did familial hypercholesterolemia subjects without CHD. Previous studies have established

TABLE 3. Clinical and Laboratory Parameters in Patients With Intermittent Claudication and Healthy Control Subjects Matched for Age, Sex, and Smoking Habits

<table>
<thead>
<tr>
<th>Variables</th>
<th>IC subjects (n = 100)</th>
<th>Control subjects (n = 100)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.7±4.5</td>
<td>63.6±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.8±3.8</td>
<td>24.7±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sedimentation rate (mm/hr)</td>
<td>12.1±10.1</td>
<td>6.7±5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>143.4±10.9</td>
<td>142.5±8.8</td>
<td>NS</td>
</tr>
<tr>
<td>Leukocyte count (10⁹/l)</td>
<td>7.5±4.5</td>
<td>6.2±1.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (10⁹/l)</td>
<td>235.7±61.4</td>
<td>235.7±60.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>108.2±51.1</td>
<td>95.2±12.2</td>
<td>&lt;0.042</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>39.3±2.6</td>
<td>39.4±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.41±1.97</td>
<td>4.60±0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Serum AST (μkat/l)</td>
<td>0.35±0.16</td>
<td>0.34±0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ALT (μkat/l)</td>
<td>0.35±0.19</td>
<td>0.31±0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Serum free thyroxine (pmol/l)</td>
<td>17.1±3.7</td>
<td>16.5±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum TSH (milliunits/l)</td>
<td>1.4±1.4</td>
<td>1.2±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td>3.73±0.70</td>
<td>3.21±0.63</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

IC, intermittent claudication; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; NS, not significant. Values are mean±SD.

p=two-tailed significance values by Mann-Whitney U test.

*Diabetics (n = 14) were excluded from this analysis.

TABLE 4. Spearman's Rank Correlations Between Lipoprotein(a) and Several Variables in Patients With Intermittent Claudication and Healthy Control Subjects Matched for Sex, Age, and Smoking Habits

<table>
<thead>
<tr>
<th>Variables</th>
<th>IC subjects</th>
<th>p</th>
<th>Control subjects</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo B</td>
<td>0.20</td>
<td>0.002</td>
<td>0.24</td>
<td>0.008</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.31</td>
<td>0.001</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>HDL chol</td>
<td>-0.11</td>
<td>NS</td>
<td>-0.10</td>
<td>NS</td>
</tr>
<tr>
<td>LDL chol</td>
<td>0.30</td>
<td>0.001</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Sed rate</td>
<td>0.22</td>
<td>0.015</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Total chol</td>
<td>0.24</td>
<td>0.009</td>
<td>0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

IC, intermittent claudication; apo, apolipoprotein; HDL, high density lipoprotein; chol, cholesterol; LDL, low density lipoprotein; sed, sedimentation; NS, not significant.

the contribution of apo(a) type was eliminated by Lp(a), probably because of the high intercorrelation (r=0.54) between these two variables.

Discussion

The present study shows a significant elevation of Lp(a) in patients with IC compared with healthy control subjects matched for sex, age, and smoking habits. The elevation of Lp(a) concentrations is mainly due to differences in the frequency distribution of apo(a) isoforms, with a preponderance of low-molecular-weight isoforms in IC subjects. Apo(a) isoforms are determined by one multiallelic gene locus on chromosome 6p that determines about 40% of the variation in the concentration of Lp(a) in plasma in whites. The study thus suggests that apo(a) polymorphism could be an important genetic risk factor for IC.

In a recently published study, it was observed that patients with familial hypercholesterolemia and CHD had a significantly higher frequency of lower-molecular-weight apo(a) isoforms and higher Lp(a) plasma concentrations than did familial hypercholesterolemia subjects without CHD. Previous studies have established...
that elevated Lp(a) levels are associated with CHD and possibly with stroke. The present study shows that elevated Lp(a) levels are also significantly associated with peripheral atherosclerosis and thus extends the evidence for Lp(a) as a possible risk factor for all major clinical types of atherosclerosis. Furthermore, logistic regression analysis shows that the increase in Lp(a) concentration in IC is not explained by differences in other major risk factors for peripheral atherosclerosis, thus showing that elevated Lp(a) is independently associated with IC. Because the IC and the healthy control subjects were matched for smoking habits, the study also suggests that elevated Lp(a) may be one explanation for the difference in risk that smokers have for developing IC.

We also found that for most of the apo(a) phenotypes, IC patients tended to have higher Lp(a) concentrations than did control subjects. For phenotypes S2 and S3, the Lp(a) concentrations in IC subjects were significantly higher than for control subjects with the same phenotypes (Table 1). This suggests either a non-genetic partial determinant of Lp(a) concentrations or other genetic differences between IC and control subjects that are unrelated to the polymorphism of apo(a). There was a weak correlation of Lp(a) with sedimentation rate and fibrinogen level; however, these correlations are too small to account for non-apo(a) isoform differences in Lp(a) concentrations.

The higher Lp(a) concentrations in IC were not explained by a concomitant high frequency of CHD in IC subjects. Lp(a) concentrations in IC subjects with (n=54) and without (n=46) CHD did not differ significantly (median values were 23.05 and 17.01 mg/dl, respectively; p=NS). There were 41 patients with intermittent claudication only, i.e., they did not have angiographic stenosis or a history of myocardial infarction, stroke, or TIA. These patients also had a significantly higher (p<0.05) median Lp(a) concentration of 15.15 mg/dl than did control subjects (11.11 mg/dl), suggesting that IC per se, independent of other clinical manifestations of atherosclerosis, is associated with high Lp(a) concentrations. Another way to study this question is to distinguish between IC and other clinical symptoms as the first manifestation of disease. In 65 patients of this study whose first symptom was IC, the median Lp(a) level (19.93 mg/dl) was significantly higher than that in control subjects (p<0.003).

Factors that may influence Lp(a) levels are certain drugs (nicotinic acid and acetylcysteine), severely reduced renal function, diabetes mellitus with microalbuminuria, and possibly liver disease. In this study none of the IC patients took lipid-lowering medication, but drugs for hypertension or angina were in common use. In IC subjects taking medication (n=79), Lp(a) concentrations did not differ compared with those who were not (n=21) (20.30 versus 19.12 mg/dl, respectively; p=NS). IC subjects with diabetes mellitus (n=14) had a great range of Lp(a) values, but their median Lp(a) value (7.43 mg/dl) did not differ from that of IC subjects without diabetes (n=86) (22.17 mg/dl, p=NS). Creatinine levels did not influence the Lp(a) concentrations in IC subjects. When grouped according to creatinine levels higher or lower than 108 µkat/l (mean value), the difference in Lp(a) concentrations (18.43 and 20.12 mg/dl, respectively) was not significant.

The Lp(a)-specific glycoprotein apo(a) has a high degree of homology to plasminogen, and its genes are closely linked on chromosome 6. Elevated Lp(a) plays a role in the fibrinolytic process. However, it has not yet been shown that Lp(a) raises the risk of developing IC. In our study, the Lp(a) concentrations did not differ significantly between IC subjects who had had a thrombotic event, such as a myocardial infarction, stroke, or TIA (n=39; Lp[a], 24.12 mg/dl) and those who had not (n=61; Lp[a], 18.06 mg/dl; p=NS).

The pathogenic mechanisms by which Lp(a) contributes to the development of atherosclerosis are not known. Lp(a) is found in athero sclerotic plaques in both coronary arteries and vein grafts, but how Lp(a) accumulates in the plaques is not clear. Foam cell formation is an important part of the atherosclerotic process. Native Lp(a), however, does not seem to stimulate foam cell formation. However, it is possible that modified Lp(a), by analogy with modified LDL, is taken up by the macrophage scavenger receptor and in this way contributes to foam cell formation. Another possibility is that Lp(a) may build aggregates that are taken up by macrophages, or that postprandially induced apo(a) stimulates lipid accumulation in macrophages.

We conclude that elevated Lp(a) concentrations are associated with IC, independent of other important risk factors for peripheral atherosclerosis such as smoking, hypertension, diabetes mellitus, elevated plasma total or LDL cholesterol, hypertriglyceridemia, increased apo B, and low HDL cholesterol concentrations. The elevation of Lp(a) in IC is explained by 1) a more unfavorable frequency distribution of apo(a) isoforms, with a higher frequency of low-molecular-weight isoforms leading to increased Lp(a) concentrations and 2) some as-yet-unknown genetic or nongenetic factors that result in higher Lp(a) levels in IC subjects with apo(a) phenotypes S2 and S3.

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