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

Jörgen 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, 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.

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 Kåre 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 Uttermann 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 Uttermann 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'-methylene-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-
ich and Schuell) by the method described by Towbin et al with the Trans-blot cell (Bio-Rad, Richmond, Calif.). Antigens were visualized on the nitrocellulose filters by a double-antibody procedure. The first antibody was a monoclonal anti-Lp(a) raised in mice. The second antibody was a rabbit anti-mouse peroxidase conjugate (Dako-Patts, Glostrup, Denmark).

With the terminology proposed by Utermann et al, the various isoforms of apo(a) have been designated according to their electrophoretic mobility on SDS-PAGE relative to that of apo B-100. “F” denotes a band faster than apo B 100, and S1, S2, S3, and S4 denote bands with progressively slower mobility than that of apo B 100.

“B” designates a band with the same mobility as apo B-100. The null type is the designation for blots showing no bands at all. The presence of two bands identifies heterozygosity, whereas the presence of only one band is consistent with homozygosity for the isoform in question or heterozygosity for the null allele (“0” in Figure 1) and the isoform. Samples from heterozygotes with phenotypes S1S2 and BS3 were run alternatively in every third lane as references (Figure 1). Standardization was performed in collaboration with Gerd Utermann (Innsbruck, Austria) and Ulrike Beisiegel (Hamburg, FRG) within the context of the European Concerted Actions Program.

Analysis of Plasma Lipids, Lipoproteins, and Apo A-I and B

Plasma lipoproteins were separated by preparative ultracentrifugation at d=1.006 kg/l into very low density lipoproteins (VLDLs), LDLs, and HDLs. In the infranatant, LDL was precipitated by phosphotungstic acid/magnesium chloride. Cholesterol and triglyceride concentrations in total plasma and lipoproteins were determined by enzymatic colorimetric methods (Monotest cholesterol CHOD-PAP and triglyceride GPO-PAP, Boehringer Mannheim, GmbH, Mannheim, FRG). Apo A-I and B were determined by rocket immunoelectrophoresis (apo A-I antiserum from Dako-Patts; apo B antiserum from Orion). The methods have been described in detail elsewhere.

Hematology and blood chemistry parameters, liver function tests, thyroid function tests, and blood glucose and fibrinogen levels were analyzed by standard procedures.

Treadmill Exercise Test

IC and control subjects rested in the supine position for 10 minutes, after which the upper arm and ankle systolic pressures were measured with a blood pressure cuff (width, 12 cm) by using a mercury manometer and a 10-MHz Doppler probe on the radial and posterior tibial arteries, respectively. The subjects walked for 1 minute at 4 km/hr on a 10% slope; thereafter they returned to the bed, and simultaneous systolic ankle and arm blood pressures were measured immediately, after 1.5 minutes, 2 minutes, and every minute for 5 minutes after the treadmill test. After 10 minutes of rest the exercise test was repeated, with measurements taken on the other leg.

A resting systolic ankle to arm pressure index ≤0.90 or a fall in systolic ankle pressure of ≥30 mm Hg immediately after exercise was considered abnormal.

Resting Blood Pressure

Systolic and diastolic blood pressures were measured in both arms by auscultation by using a mercury manometer 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 statistical differences in variables between the IC and control groups. The Kruskal-Wallis and χ² 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 associations 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 dependent variable and then sequentially adding age, smoking, 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 Committee 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 frequency distributions of Lp(a) were skewed toward low concentrations of Lp(a), but this trend was less pronounced in IC than in control subjects (Table 1 and Figure 2). The frequency distribution of apo(a) phenotypes 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 control 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 cholesterol concentrations were significantly higher and HDL concentrations were lower in IC subjects.
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>...</td>
<td>0</td>
</tr>
<tr>
<td>BS4</td>
<td>101.34</td>
<td>1</td>
</tr>
<tr>
<td>S1</td>
<td>76.30 (46.61–121.46)</td>
<td>5</td>
</tr>
<tr>
<td>S1S2</td>
<td>322.20</td>
<td>1</td>
</tr>
<tr>
<td>S1S3</td>
<td>...</td>
<td>0</td>
</tr>
<tr>
<td>S1S4</td>
<td>124.24</td>
<td>1</td>
</tr>
<tr>
<td>S2</td>
<td>60.70 (4.07–205.96)</td>
<td>25</td>
</tr>
<tr>
<td>S2S3</td>
<td>77.97 (54.29–101.64)</td>
<td>2</td>
</tr>
<tr>
<td>S2S4</td>
<td>19.39 (6.18–83.86)</td>
<td>7</td>
</tr>
<tr>
<td>S3</td>
<td>30.18 (1.68–113.72)</td>
<td>13</td>
</tr>
<tr>
<td>S3S4</td>
<td>11.79 (1.68–29.58)</td>
<td>10</td>
</tr>
<tr>
<td>S4</td>
<td>12.41 (3.20–36.33)</td>
<td>24</td>
</tr>
<tr>
<td>&quot;0&quot; (null)</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.

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-

FIGURE 2. Frequency distribution of plasma lipoprotein(a) (Lp[a]) concentrations in subjects with intermittent claudication and healthy controls matched for sex, age, and smoking habits. Because each group consists of 100 subjects, the y axis indicates both the numbers and percentages.
projected plasma fibrinogen, LDL cholesterol, total plasma lipid variables

IC subjects Control subjects $p$
---
Total cholesterol* $5.63±1.05$ $5.26±0.87$ $<0.011$
Total triglycerides* $1.72±1.15$ $1.11±0.54$ $<0.00001$
VLDL cholesterol* $0.56±0.55$ $0.32±0.23$ $<0.00001$
VLDL triglycerides* $1.25±1.08$ $0.73±0.48$ $<0.00001$
LDL cholesterol* $4.06±0.96$ $3.73±0.81$ $<0.009$
HDL cholesterol* $1.23±0.29$ $1.17±0.29$ $<0.00001$
Apolipoprotein A-I* $1.39±0.30$ $1.25±0.29$ $<0.003$
Apolipoprotein B* $1.23±0.19$ $1.31±0.15$ $<0.0003$

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.

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 6 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 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>$&lt;0.00001$</td>
</tr>
<tr>
<td>VLDL cholesterol*</td>
<td>0.56±0.55</td>
<td>0.32±0.23</td>
<td>$&lt;0.00001$</td>
</tr>
<tr>
<td>VLDL triglycerides*</td>
<td>1.25±1.08</td>
<td>0.73±0.48</td>
<td>$&lt;0.00001$</td>
</tr>
<tr>
<td>LDL cholesterol*</td>
<td>4.06±0.96</td>
<td>3.73±0.81</td>
<td>$&lt;0.009$</td>
</tr>
<tr>
<td>HDL cholesterol*</td>
<td>1.23±0.29</td>
<td>1.17±0.29</td>
<td>$&lt;0.00001$</td>
</tr>
<tr>
<td>Apolipoprotein A-I*</td>
<td>1.39±0.30</td>
<td>1.25±0.29</td>
<td>$&lt;0.003$</td>
</tr>
<tr>
<td>Apolipoprotein B*</td>
<td>1.23±0.19</td>
<td>1.31±0.15</td>
<td>$&lt;0.0003$</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.

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

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/m$^2$)</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>$&lt;0.00001$</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 (10$^9$/l)</td>
<td>7.5±4.5</td>
<td>6.2±1.7</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Platelet count (10$^9$/l)</td>
<td>235.7±61.4</td>
<td>235.7±60.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine ($\mu$mol/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 ($\mu$kat/l)</td>
<td>0.35±0.16</td>
<td>0.34±0.11</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ALT ($\mu$kat/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>$&lt;0.00001$</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>0.68</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>0.68</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.
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 nongenetic 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 angina pectoris 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<0.005).

Factors that may influence Lp(a) levels are certain drugs (nicotinic acid and acetylsalicylate), 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 their genes are closely linked on chromosome 6.38,43 It is therefore possible that Lp(a) could play a role in the fibrinolytic process. However, it has not yet been shown that Lp(a) plays a role in clinical thrombogenesis. In our study, the Lp(a) concentrations did not differ significantly between IC subjects who had had a thrombogenic 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 atherosclerotic plaques in both coronary arteries and vein grafts,44,45 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.46 However, it is possible that modified Lp(a), by analogy with modified LDL cholesterol, is taken up by the macrophage scavenger receptor and in this way contributes to foam cell formation.47 Another possibility is that Lp(a) may build aggregates that are taken up by macrophages,48 or that postprandially induced apo(a) stimulates lipid accumulation in macrophages.49

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.

Acknowledgments

The monoclonal apo(a) antibodies were kindly supplied by Gerd Utermann, Innsbruck, Austria. We thank nurses Inga Friden and Elisabeth Svedberg for their skillful handling of blood samples and patients. We are also indebted to Ms. Gitte Gilstup Nielsen, Ms. Anette Stenderup, and Ms. Lene Trudsoe for excellent technical assistance.

References

10. Utermann G, Kraft HG, Menzel HJ, Hopferwieser T, Seitz C: Genetics of the quantitative Lp(a) lipoprotein trait: I. Relation of Lp(a) glycoprotein phenotypes to Lp(a) lipoprotein concentrations in plasma. Hum Genet 1987; 84:41–46
15. Dahlén G, Berg K, Gillnäs T, Ericsson C: Lp(a) lipoprotein/pre-
16. Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Quinci GB: Lipoprotein (a) and the risk for myocardial infarc-
ersclerosis: Dependence on serum LDL levels. Atherosclerosis 1986;62:249–257
21. Sandkamp M, Funke H, Schulte H, Köhler E, Assmann G: Lipoprotein(a) is an independent risk factor for myocardial infarc-
22. Rosengren A, Wilhelmson L, Eriksson K, Rösberg B, Wedel H: Lp(a) levels in patients with coronary heart disease: A prospective case-
control study in a general population sample of middle aged men. BMJ 1990;301:1248–1251
glund L, Bondjers G: Apolipoprotein(a) and ischaemic heart dis-
case in familial hypercholesterolaemia. Lancet 1990;335:1360–1363
25. Seed M, Hoppichler F, Reaveley D, McCarthy S, Thompson GR, Boerwinkle E, Utermann G: Relation of serum lipoprotein(a)
26. Kühnlein J, Jürgens G: A dominant role of lipoprotein (a) in the investigation and evaluation of parameters indicating the develop-
29. Rose GA, Blackburn H, Gillum RF, Prineas RJ: Annex 6: Cardio-
vascular Survey Methods, ed 2. Geneva, Switzerland, World Health Organization, 1982
32. Neville DM: Molecular weight determination of protein–dodecyl
33. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Proce-
dure and some applications. Proc Natl Acad Sci U S A 1979;76: 4350–4354
34. Kraft H-G, Dieppling H, Hoye E, Utermann G: Lp(a) phenotyping
by immunoblotting with polyclonal and monoclonal antibodies. Arteriosclerosis 1986;12:212–216
36. Laing SP, Greenhalgh RM: Standard exercise test to assess periph-
eral arterial disease. BMJ 1980;281:13–16
40. Gavish D, Breslow JL: Lipoprotein(a) reduction by N-acetylys-
42. Jenkins AJ, Steele JS, Janus ED, Best JD: Increased plasma apo-
lipoprotein(a) levels in IDDM patients with microalbuminuria. Diabetes 1991;40:787–790
47. Haberland ME, Flesa G, Scarno AM, Fogelman AM: Modification of Lp(a) by malondialdehyde leads to avid uptake by human mono-
49. Bernt TF, Innerarity TL, Psycho RE, Rea SC Jr, Weisgraber KH, Mahley RW: Fat feeding in humans induces lipoproteins of density less than 1.006 that are enriched in apolipoprotein (a) and that cause lipid accumulation in macrophages. J Clin Invest 1986;77: 622–630
Significant association between low-molecular-weight apolipoprotein(a) isoforms and intermittent claudication.

J Mölgaard, I C Klausen, C Lassvik, O Faergeman, L U Gerdes and A G Olsson

doi: 10.1161/01.ATV.12.8.895

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/12/8/895

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/