Serum Lipoproteins and Hemostatic Function in Intermittent Claudication

J. Johansson, N. Egberg, H. Johnsson, L.A. Carlson

Normoglycemic men with intermittent claudication (n=41), mean age of 63 years, and sex-, age-, body mass index-, and smoking habit–matched controls (n=75) were compared for plasma lipoprotein and hemostatic variables. The patients had significantly lower levels of large high-density lipoprotein (HDL) particles (HDL_{L2a}, HDL_{L2b}, and HDL_{L3}) and elevated lipoprotein(a) [Lp(a)] concentrations than the control subjects. Of the hemostatic variables, plasminogen activator inhibitor-1 (PAI-1), plasma antithrombin, plasma fibrinogen, and plasma α2-antiplasmin concentrations were significantly elevated in patients. In intermittent claudication patients Lp(a) correlated significantly with activation of the coagulation system, ie, with the levels of plasma fibrinogen and urinary fibrinopeptide A. No correlations between the values for Lp(a) and PAI-1 or plasma α2-antiplasmin were seen. The PAI-1 activity showed significant univariate correlations to the levels of HDL_{L2}, HDL_{L3}, and very-low-density lipoprotein triglycerides, of which the positive correlation to HDL_{L2} persisted in multivariate analysis (r=0.48, P<.001). Independent characteristics for intermittent claudication estimated by multiple regression analysis were PAI-1, plasma fibrinogen, and HDL_{L2}, with a combined R² of 0.36. The intermittent claudication subgroup that was being treated with β-blockers/thiazides had a higher frequency of coronary heart disease compared with other patients. In addition, the patients taking β-blockers/thiazides had elevated triglyceride concentrations, lower HDL cholesterol with a size shift toward smaller particles, and a tendency toward raised PAI-1 and plasma α2-antiplasmin levels compared with the patient group that did not take these medications. (Arterioscler Thromb. 1993;13:1441-1448.)

KEY WORDS • intermittent claudication • lipoproteins • hemostasis function • triglyceride • HDL particle size subclasses • plasma fibrinogen • plasminogen activator inhibitor-1

Pronounced arteriosclerosis of the lower part of the aorta, the iliac arteries, and the arteries of the lower limbs affects walking capacity, leads to intermittent claudication, and may eventually lead to gangrene of the limb. Subjects with intermittent claudication also have an increased incidence of coronary heart disease (CHD) and cerebrovascular disease.1,2 A number of risk factors for intermittent claudication have been suggested, of which cigarette smoking and diabetes mellitus have been shown to be of the greatest importance.3,4 The roles of hypertension and serum lipoproteins in the genesis of intermittent claudication are less convincing than in the development of CHD.5,6 But low concentrations of high-density lipoprotein cholesterol (HDL-C) have been shown to be a risk factor for intermittent claudication.6,7 Two recent studies indicate that the inverse relation of HDL to atherosclerotic disease in the coronary arteries is accounted for by low levels of the largest HDL particles.8,9 Whether such a relation also applies to peripheral arteriosclerosis, ie, intermittent claudication, has not been reported.

An imbalance of the hemostatic systems also appears to be associated with intermittent claudication. Plasma fibrinogen concentration is increased in patients with intermittent claudication.10 A continuous intravascular deposition of fibrin in vivo counterbalanced by an incessant fibrinolytic process is likely. Recent studies suggest that this balance is affected by lipoproteins, particularly the triglyceride-rich ones.11,12

In the present study lipoproteins and hemostatic variables were analyzed in patients with intermittent claudication. Our objective was twofold: first, to elucidate possible links between hemostatic function and lipoprotein metabolism, and second, to evaluate the explanatory power for intermittent claudication of both hemostatic and lipoprotein variables.

Methods

Patients

Forty-one normoglycemic men 63±4 (mean±SD) years of age who had been consecutively referred to the peripheral vascular unit at Karolinska Hospital during 1989 through 1990 because of clinical symptoms of intermittent claudication participated in the study. Patients and control subjects (vide infra) were informed about the study and their consent was obtained. Patients with rest pain or ulcers were excluded. None of the patients were taking lipid-lowering medication, and no major medical event had occurred during the 6 months before referral. All participants were examined by the same physician (Dr J. Johansson). The clinical characteristics of the subjects were evaluated by a structured interview protocol. A Swedish translation of Rose's

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questionnaire for intermittent claudication and angina pectoris was used.\textsuperscript{13} Sixteen patients had hypertension and 8 had angina pectoris. A history of myocardial infarction was present in 3 patients and coronary bypass surgery had been performed on 3 subjects. Six patients took selective and 6 took nonselective \(\beta\)-blockers. Four patients took thiazide diuretics, two of whom were also on \(\beta\)-blockers. Another 10 patients regularly took some type of medication. Only 13 patients were free from hypertension or CHD and did not take regular medication. Of the 14 patients who took \(\beta\)-blockers/thiazides, 8 had hypertension, 3 had angina pectoris, 2 had survived a myocardial infarction, and another 2 had been treated for angina pectoris with bypass surgery. The clinical diagnosis of patients and control subjects was confirmed by pulse plethysmography of the first toe from both sides and distal blood pressure measurements.\textsuperscript{14}

**Selection of Control Subjects**

To every index patient an age- and sex-matched control subject was randomly chosen from the Stockholm County population. To avoid the possibly confounding influences of smoking and obesity on the concentrations of the measured lipoprotein and hemostasis variables, the index patients were also matched with a second control group not only for sex and age but also for smoking and body mass index (BMI; kg/m\(^2\)). For each of the 41 patients, 10 men, born the same day as the patient, were randomly chosen from the population register (L-data) after exclusion of subjects with a history of hospital treatment during the last 5 years or presence of chronic disease including diabetes mellitus. A questionnaire inquiring about smoking habits, height, weight, status of health, and regular use of medication was sent to each of these 10 men. Mean response rate was 7 men (maximum, 10; minimum, 4). One of the men was randomly matched to the index patient to represent a “normal” subject in the general population, i.e., matched only for age and sex. From the same original group a case-control subject for the index patient was picked by matching not only for sex and age but also for smoking habits and BMI. By this procedure 7 of the 41 randomly chosen normal subjects became identical with 7 of the selected control subjects. Lipoprotein analysis (see below) was done on all included subjects except for the lipoprotein(a) \([Lp(a)]\) analysis, which was done on frozen serum from the last 27 patients and their sex-, age-, smoking habit-, and BMI-matched case-control subjects. Tests on hemostasis were consecutively and randomly performed in a subsample (33 control subjects and 24 patients).

**Lipoproteins**

Venous blood was drawn from the patients between 8 and 9 AM after fasting overnight. HDL particle size subclass analysis was performed on ice-cooled plasma by a combination of ultracentrifugation and precipitation techniques as previously described,\textsuperscript{17} and cholesterol\textsuperscript{18} and triglyceride\textsuperscript{19} concentrations were determined in serum and in each of the VLDL, LDL, and HDL fractions. Lp(a) analysis was performed in one run on serum that had been stored at \(-70^\circ\)C by using a commercial immunoradiometric assay (Pharmacia, Uppsala, Sweden).

**Hemostatic Variables**

Nine parts venous blood was drawn into one part trisodium citrate solution, 0.129 mol/L. Blood for determination of \(\beta\)-thromboglobulin and fibrinopeptide A was anticoagulated according to the following references. Fibrinogen determinations were performed by a polymerization rate assay.\textsuperscript{20} Fibrinopeptide A was determined in plasma and in urine by means of a radioimmunoassay\textsuperscript{21} using reagents from IMCO, Stockholm, Sweden. The plasminogen activator inhibitor-1 (PAI-1) activity was determined by a functional spectrophotometric method\textsuperscript{22} by using a kit from Biopool AB, Umeå, Sweden. \(\alpha_2\)-Antiplasmin was determined with a spectrophotometric assay\textsuperscript{23} by using kits from KabiPharma, Stockholm, Sweden. Thrombin-antithrombin complex was determined by an enzyme-linked immunosorbent assay\textsuperscript{24} (ELISA) kit (Enzydiagnos TAT Micro; Behringwerke AG, Marburg, FRG). \(\beta\)-Thromboglobulin in urine was determined by using a modification of a commercially available kit (Amersham, UK). The labeled antigen and the antiserum were diluted five times. von Willebrand factor antigen was determined by means of a commercial ELISA\textsuperscript{25} (Asserachrom vWF, Diagnostica Stago, Asnieres, France). Protein C was determined by a functional amidolytic assay\textsuperscript{26} (Coamate, Protein C, Chromogenix AB, Mölndal, Sweden). Orosomucoid was determined by a routine nephelometric immunochemical assay.

**Statistics**

All statistical calculations were performed with a statistical computer program (STATGRAPHICS, Statistical Graphics Corporation, Inc, Rockville, Md). Mean values and standard deviations were calculated by conventional analysis. Individual values of skewed variables were fitted by logarithm to near-normal distribution. An unpaired Student's \(t\) test was used for group comparisons. Univariate correlations were calculated with the Spearman rank test and multivariate correlations by partial correlation analysis. In the multiple stepwise regression analysis the variable with the highest partial correlation coefficient was entered at each step until no variable remained with a probability value of less than .05.

**Results**

**Matching of Controls**

To our surprise, the smoking habits and the BMI of the randomly chosen normals and the selected smoking habit- and BMI-matched case-control subjects turned out to be virtually identical. The clinical characteristics given in Table 1 were insignificantly different between the normal subjects and the case-control subjects.
Patients with intermittent claudication had higher serum triglyceride levels and higher VLDL triglyceride concentrations than control subjects, which was accounted for by the β-blocker/thiazide–treated patient subgroup. This subgroup also showed a lower HDL-C concentration and a higher total cholesterol value (Table 2).

**Lipoproteins**

Cholesterol and triglycerides in the major lipoprotein fractions. Patients with intermittent claudication had higher serum triglyceride levels and higher VLDL cholesterol and VLDL triglyceride concentrations than control subjects, which was accounted for by the β-blocker/thiazide–treated patient subgroup. This subgroup also showed a lower HDL-C concentration and a higher total cholesterol value (Table 2).

Lp(a). The Lp(a) concentration was significantly higher for patients, mostly as a result of the elevated levels in the β-blocker/thiazide–treated patient subgroup. The Lp(a) concentration was correlated to plasma fibrinogen in patients (r=0.46, P<0.05) but not in control subjects. The urine fibrinopeptide A level was also positively correlated with the Lp(a) concentration (r=0.41, P<0.05), but no clear correlation for patients could be established because we had only seven observations of both variables in the patient group. No significant correlations between Lp(a) and variables describing fibrinoelastic capacity, ie, PAI-1 and plasma α2-antiplasmin, were found for either patients or controls.

**HDL subclass concentrations.** The total HDL protein level was decreased in patients with intermittent claudication compared with control subjects because of significantly lower patient concentrations of the three subclasses containing the largest HDL particles, ie, HDL$_2^c$, HDL$_2^a$, and HDL$_3^a$ (Table 3). The β-blocker/thiazide–treated patient subgroup had elevated HDL$_2^c$ and decreased HDL$_3^a$ levels compared with both the other patients and control subjects.

**Hemostatic Variables**

Because of the unbalanced prevalence of current smokers in the control and patient groups, for the data on which analyses were performed on hemostatic variables the two groups were divided into smokers and nonsmokers, and the concentrations of the tested variables were compared. Of the hemostatic variables, only the plasma fibrinogen concentration in the control group differed significantly between smokers and nonsmokers (3.48 g/L and 2.91 g/L, respectively; P<0.01). For the patients, the corresponding values were 4.01 g/L for smokers and 3.58 g/L for nonsmokers (not significant [NS]).

**Platelets.** Platelet counts were the same for both patients and control subjects. The urinary excretion of β-thromboglobulin–like material, a marker for in vivo platelet activation, was significantly higher in patients (Table 4), which was mostly accounted for by the patients not treated with β-blockers/thiazides.

**Coagulation system activation.** The plasma fibrinogen concentration was significantly higher in the patient group than in the control group (Table 4). The von Willebrand factor and protein C levels were the same in both groups. No group differences for the fibrinopeptide A concentration in plasma or urine were found. The thrombin-antithrombin complex levels were not significantly different between patients and control subjects, though there was a tendency toward higher values in the patients.

**Fibrinolytic variables.** The PAI-1 and α2-antiplasmin values were significantly higher in patients, indicating an impaired fibrinoelastic capacity (Table 4). These elevations were mainly accounted for by the β-blocker/thiazide subgroup.
TABLE 2. Cholesterol and Triglyceride Concentrations and the Major Lipoprotein Fractions in Patients and Control Subjects

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Control Subjects (n=75)</th>
<th>Patients (n=41)</th>
<th>Without β-Blockers/Thiazides (n=27)</th>
<th>With β-Blockers/Thiazides (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>6.60 (1.14)</td>
<td>6.90 (1.49)</td>
<td>6.68 (1.55)</td>
<td>7.32 (1.32)*</td>
</tr>
<tr>
<td>TG</td>
<td>1.57 (0.65)</td>
<td>2.11 (1.48)†</td>
<td>1.78 (1.57)</td>
<td>2.75 (1.05)††</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>0.59 (0.35)</td>
<td>0.83 (0.63)*</td>
<td>0.66 (0.57)</td>
<td>1.16 (0.63)††</td>
</tr>
<tr>
<td>TG</td>
<td>0.96 (0.53)</td>
<td>1.48 (1.33)†</td>
<td>1.19 (1.44)</td>
<td>2.02 (0.94)††</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>4.57 (0.87)</td>
<td>4.74 (1.34)</td>
<td>4.63 (1.37)</td>
<td>4.94 (1.31)</td>
</tr>
<tr>
<td>TG</td>
<td>0.40 (0.12)</td>
<td>0.42 (0.12)</td>
<td>0.39 (0.11)</td>
<td>0.46 (0.11)</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>1.40 (0.29)</td>
<td>1.30 (0.29)</td>
<td>1.35 (0.32)</td>
<td>1.2 (0.2)*</td>
</tr>
<tr>
<td>TG</td>
<td>0.15 (0.03)</td>
<td>0.18 (0.05)</td>
<td>0.15 (0.04)</td>
<td>0.17 (0.05)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>228 (311)</td>
<td>343 (318)*</td>
<td>270 (210)</td>
<td>516 (461)</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein. HDLs were separated by gradient gel electrophoresis, and the protein concentrations were analyzed within defined particle size intervals in milligrams per milliliter plasma. See "Methods" for details. Values are mean (SD).

*P<.05, †P<.01, ‡P<.001 all patients compared with control subjects.
§P<.05 patients with β-blockers/thiazides compared with patients without β-blockers/thiazides.

Characteristics of Patients With Intermittent Claudication

Multiple stepwise regression analysis, with intermittent claudication as the dependent variable and VLDL triglycerides, LDL cholesterol (LDL-C), HDL2b, HDL3a, HDL3b, PAI-1 activity, plasma fibrinogen, and plasma α2-antiplasmin as independent variables, showed that 36% (adjusted R2 = .36) of the intermittent claudication cases could be explained by PAI-1, plasma fibrinogen, and HDL3a. Their independent contributions (adjusted R2) were .20, .11, and .05, respectively. The corresponding values, ie, from the group that included only patients not treated with β-blockers and/or thiazides, were .20, .07, and .06, respectively.

Relations Between Various Lipoproteins and Hemostatic Function Variables

The concentrations of VLDL triglycerides, LDL-C, HDL2b, and HDL3a were chosen to represent VLDL, LDL, and large and small HDL particles, respectively, and correlated to PAI-1 activity, plasma fibrinogen, and plasma orosomucoid levels (Table 5).

TABLE 3. HDL Subclass Concentrations in Intermittent Claudication Patients and Control Subjects

<table>
<thead>
<tr>
<th>HDL</th>
<th>Control Subjects (n=75)</th>
<th>All (n=41)</th>
<th>Without β-Blockers/Thiazides (n=27)</th>
<th>With β-Blockers/Thiazides (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2b</td>
<td>0.23 (0.12)</td>
<td>0.18 (0.10)*</td>
<td>0.21 (0.10)</td>
<td>0.14 (0.07)§</td>
</tr>
<tr>
<td>2a</td>
<td>0.40 (0.11)</td>
<td>0.36 (0.11)*</td>
<td>0.37 (0.12)</td>
<td>0.33 (0.09)*</td>
</tr>
<tr>
<td>3a</td>
<td>0.52 (0.11)</td>
<td>0.47 (0.09)†</td>
<td>0.45 (0.09)†</td>
<td>0.49 (0.10)</td>
</tr>
<tr>
<td>3b</td>
<td>0.35 (0.08)</td>
<td>0.37 (0.09)</td>
<td>0.34 (0.07)</td>
<td>0.42 (0.09)§</td>
</tr>
<tr>
<td>3c</td>
<td>0.19 (0.07)</td>
<td>0.20 (0.06)</td>
<td>0.18 (0.05)</td>
<td>0.22 (0.06)</td>
</tr>
<tr>
<td>Total</td>
<td>1.70 (0.28)</td>
<td>1.57 (0.23)*</td>
<td>1.55 (0.23)*</td>
<td>1.59 (0.21)</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein. HDLs were separated by gradient gel electrophoresis, and the protein concentrations were analyzed within defined particle size intervals in milligrams per milliliter plasma. See "Methods" for details. Values are mean (SD).

*P<.05, †P<.01 all patients compared with control subjects.
§P<.05 patients with β-blockers/thiazides compared with patients without β-blockers/thiazides.
Univariate analysis (Table 5) showed a highly significant positive correlation between the HDL$_3$ level and PAI-1 activity. In contrast, the HDL$_2$ concentration and PAI-1 were significantly negatively correlated. The values for HDL$_2$, and HDL$_3$ also showed positive and negative correlations, respectively, to VLDLs. The level of VLDL triglycerides and PAI-1 activity were positively correlated. Plasma fibrinogen concentration and plasma orosomucoid were highly significantly positively correlated. The level of VLDL triglycerides and PAI-1 were significantly negatively correlated. The level of HDL$_3$ and VLDL triglycerides correlated highly significantly as well.

In partial correlation analysis (Table 5), HDL$_3$ and PAI-1 remained highly significantly correlated when the concentrations of VLDL triglycerides, HDL$_2$, and plasma fibrinogen were included in the model. Under similar conditions, HDL$_3$ and VLDL triglycerides correlated highly significantly as well.

The Figure shows the regression curves and the corresponding equations for the concentrations of VLDL triglycerides, HDL$_2$, and HDL$_3$ on PAI-1 activity. The HDL$_3$ concentration was highly significantly positively correlated to the PAI-1 activity ($R^2=.38, P<.001$) for both patients and control subjects ($R^2=.47$ and .31, respectively; both $P<.001$). The HDL$_2$ level was significantly and inversely correlated to the PAI-1 activity ($R^2=.23, P<.01$) in patients and control subjects (both $R^2=.21$, $P<.05$ and $P<.01$, respectively). The correlation between VLDL triglyceride levels and PAI-1 activity became significant after removal of one outlier with a VLDL triglyceride concentration above 7 mmol/L ($R^2=.16, P<.05$). This correlation was limited to the patient group and was not found in the control group ($R^2=.22, P<.05$ and $R^2=.07$, NS, respectively).

**Discussion**

The genesis of atherosclerosis is multifactorial, and intermittent claudication, which is usually associated with pronounced atherosclerosis, is a late symptom of that process. It is thus not surprising that subjects with established intermittent claudication also frequently have other manifestations of atherosclerosis and concomitant treatment for these diseases. The subjects in the patient group who were being treated with $\beta$-blockers (selective or unselective) or thiazides predictably had a greater incidence of CHD (angina pectoris, a previous myocardial infarction, or bypass surgery) and hypertension compared with the patients not treated with these agents. Differences between these two groups may thus be ascribed either to the more severe disease of the patients on medication, the $\beta$-blocker/thiazide treatment itself, or to both factors. This cannot directly be solved by a case-control study like ours. However, the results of the multiple regression analysis for presence of intermittent claudication was basically the same regardless of whether the patients treated with $\beta$-blockers/thiazides were included. This suggests that the results are related to the disease and that they are not secondary to treatment.

The new findings of the present study were that normoglycemic intermittent claudication patients, when compared with age-, sex-, BMI-, and smoking habit–matched controls, had (1) lower levels of large HDL particle size subclasses, ie, HDL$_2b$; HDL$_2c$, and HDL$_3b$; (2) higher PAI-1 activity; and (3) a highly significant positive correlation between PAI-1 and the concentration of HDL$_3$. Finally, (4) the Lp(a) concentration, which was elevated in patients compared with control subjects, correlated significantly to activation of the coagulation system (plasma fibrinogen and urine fibrinopeptide) but not to variables describing fibrinolytic capacity (PAI-1 and plasma $\alpha_2$-antiplasmin).

The protein concentrations of the particle size–defined HDL subclasses HDL$_2b$, HDL$_2c$, and HDL$_3$ were

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**Table 4. Hemostasis Function Tests In Patients With Intermittent Claudication and Control Subjects**

<table>
<thead>
<tr>
<th>Hemostasis Variable</th>
<th>Control Subjects (n=33)</th>
<th>All (n=24)</th>
<th>Without $\beta$-Blockers/Thiazides (n=15)</th>
<th>With $\beta$-Blockers/Thiazides (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine $\beta$-thromboglobulin, pg/mg creatinine</td>
<td>66 (31) (n=23)</td>
<td>107 (88)*  (n=17)</td>
<td>125 (112)* (n=9)</td>
<td>88 (55) (n=7)</td>
</tr>
<tr>
<td>Plasma fibrinogen, g/L</td>
<td>3.1 (0.5)</td>
<td>3.8 (0.8)$\dagger$</td>
<td>3.8 (0.8)$\dagger$</td>
<td>3.7 (0.8)$\dagger$</td>
</tr>
<tr>
<td>Plasma fibrinopeptide A, nmol/L</td>
<td>2.3 (1.9)</td>
<td>2.6 (2.1)</td>
<td>2.2 (1.2)</td>
<td>3.5 (3.1)</td>
</tr>
<tr>
<td>Urine fibrinopeptide A, ng/mg creatinine</td>
<td>1.6 (1.2) (n=23)</td>
<td>1.2 (1.0) (n=17)</td>
<td>1.2 (1.2) (n=9)</td>
<td>1.1 (0.8) (n=7)</td>
</tr>
<tr>
<td>Plasma von Willebrand factor, U/mL</td>
<td>1.3 (0.6)</td>
<td>1.5 (0.6)</td>
<td>1.6 (0.7)</td>
<td>1.4 (0.4)</td>
</tr>
<tr>
<td>Plasma protein C, U/mL</td>
<td>1.1 (0.2)</td>
<td>1.0 (0.2)</td>
<td>1.0 (0.2)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>Plasma thrombin-antithrombin complex, % of normal</td>
<td>3.0 (1.9) (n=18)</td>
<td>4.6 (5.2) (n=19)</td>
<td>5.5 (6.6) (n=11)</td>
<td>3.4 (2.2) (n=7)</td>
</tr>
<tr>
<td>Plasminogen activator Inhibitor-1, U/mL</td>
<td>14 (10)</td>
<td>22 (14)$\dagger$</td>
<td>20 (14)</td>
<td>25 (13)$\dagger$</td>
</tr>
<tr>
<td>Plasma $\alpha_2$-antiplasmin, U/mL</td>
<td>1.02 (0.12)</td>
<td>1.11 (0.15)*</td>
<td>1.08 (0.14)</td>
<td>1.15 (0.18)*</td>
</tr>
<tr>
<td>Plasma orosomucoid, g/L</td>
<td>0.84 (0.28)</td>
<td>1.10 (0.32)$\dagger$</td>
<td>1.03 (0.30) (n=11)</td>
<td>1.27 (0.34) (n=5)</td>
</tr>
</tbody>
</table>

Values are given as mean (SD).

$*P<.05, \dagger P<.01, \ddagger P<.001$ all patients compared with control subjects.

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![Image](http://atvb.ahajournals.org/Downloaded from)
significantly lower in the patient group. The intermittent claudication patients not taking β-blockers/thiazides had only a lowered HDL₄ value. The apolipoprotein composition of various HDL subclasses may influence the capacity of reverse cholesterol transport and atherosclerosis development. Subclasses HDL₂₅ and HDL₄ contain lipoprotein particles with apolipoprotein (apo) A-I without apo A-II (Lp-A-I), whereas lipoprotein particles with both apo A-I and apo A-II are distributed along the entire HDL particle size range. The so-called atheroprotective effect associated with HDL₃, contain lipoprotein particles with apolipoprotein particles with both apo A-I and apo A-II are distributed along the entire HDL particle size range. The intermittent claudication patients not taking β-blockers/thiazides had only a lowered HDL₄ value. The capacity of reverse cholesterol transport and atherosclerosis development may influence this relation. Further studies are needed to discern the pathophysiological relevance of these findings.

The Lp(a) levels of the patients were significantly higher than those of the control subjects, a finding that confirms the results of two recent studies. A link between Lp(a) and the fibrinolytic system has been considered likely because of the high degree of homology between apo(a), the glycoprotein of Lp(a), and plasminogen. No correlations were found, however, between Lp(a) and the fibrinolytic index variables PAI-1 and plasma α₂-antiplasmin. To our surprise, the Lp(a) levels correlated positively to an activation of the coagulation system, ie, to the plasma fibrinogen and urine fibrinopeptide A concentrations. The positive correlation between the values for Lp(a) and plasma fibrinogen was found for patients but not for control subjects, suggesting that the presence of widespread atherosclerosis may influence this relation. Further studies are needed to discern the pathophysiological relevance of these findings.

Elevated plasma fibrinogen has been demonstrated in case-control studies on patients with intermittent claudication and prospectively for CHD. Our study shows that the intermittent claudication patients, for a given HDL₄ level, increased plasma fibrinogen concentrations were found, however, between Lp(a) and the fibrinolytic index variables PAI-1 and plasma α₂-antiplasmin. To our surprise, the Lp(a) levels correlated positively to an activation of the coagulation system, ie, to the plasma fibrinogen and urine fibrinopeptide A concentrations. The positive correlation between the values for Lp(a) and plasma fibrinogen was found for patients but not for control subjects, suggesting that the presence of widespread atherosclerosis may influence this relation. Further studies are needed to discern the pathophysiological relevance of these findings.

**TABLE 5. Univariate and Partial Correlation Coefficient Analyses Between Various Lipoprotein and Hemostasis Variables for Intermittent Claudication Patients and Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>PAI-1</th>
<th>Plasma Fibrinogen</th>
<th>VLDL-TG</th>
<th>HDL₂₅</th>
<th>HDL₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate analysis</td>
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<td>VLDL-TG</td>
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<td>HDL₂₅</td>
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<td>-.06</td>
<td>-.46†</td>
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<tr>
<td>HDL₄</td>
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<td>.60‡</td>
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<td>.35*</td>
<td>-.00</td>
<td>-.03</td>
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<td>Partial correlation analysis</td>
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<td>HDL₄</td>
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<td>-.27</td>
<td>.41†</td>
<td>-.28*</td>
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PAI-1 indicates plasminogen activator inhibitor-1; VLDL-TG, very-low-density lipoprotein triglycerides; HDL₄, high-density lipoprotein. Univariate correlation coefficients were obtained by using the Spearman rank test. In the partial correlation the relationship between two variables was calculated while the covariating effects by the other included variables were controlled. For control subjects, n=33 and for patients, n=24.

*P<.05, †P<.01, ‡P<.001.
Regression curves for the concentrations of very-low-density lipoprotein triglycerides (VLDL-TG; top), high-density lipoprotein triglycerides (HDLb; center), and HDLc (HDLb; bottom) on the plasminogen activator inhibitor-1 (PAI-1). Equations are as follows: for VLDL-TG, \( y = 0.021x + 0.861 \); \( R^2 = 0.23 \) (P < 0.01); and for HDLb, \( y = 0.004x + 0.276 \); \( R^2 = 0.38 \) (P < 0.001).

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