Hypertriglyceridemia and Regulation of Fibrinolytic Activity

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A relation between elevated triglyceride (TG) levels and alterations of the fibrinolytic system has been recognized in studies of patients with coronary heart disease. In this investigation, the total fibrinolytic activity and the levels of specific components of the fibrinolytic system were evaluated in plasma samples from a highly selected group of patients with type IV hyperlipoproteinemia before and after a dietary treatment aimed at reducing TG levels. The fibrinolytic response of type IV patients was comparable to that of normolipidemic subjects, whereas tissue-type plasminogen activator antigen levels before and after venous occlusion (p<0.01) and resting plasminogen activator inhibitor-1 (PAI-1) antigen (p<0.01) and activity (p<0.01) were significantly higher in hypertriglyceridemic subjects compared with controls. After dietary treatment, a 22% reduction in TG levels was attained in type IV patients, with no appreciable modification of fibrinolytic parameters. The analysis of the single-patient data revealed a tendency toward normalization of PAI-1 levels only in those patients who showed a TG reduction ≥20%. Very low density lipoproteins (VLDLs) from both normal and type IV patients concentration-dependently stimulated PAI-1 release by endothelial cells and HepG2 cells, with the effect of VLDL from type IV patients being more pronounced on HepG2 cells. The release of PAI-1 induced by VLDL in competent cells may thus account for the elevated levels of this antifibrinolytic protein that occur in hypertriglyceridemic patients.

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In recent years, the concept of elevated cholesterol and low density lipoprotein (LDL) levels as risk factors for coronary heart disease (CHD) has been widely accepted.1 In contrast, the evidence concerning hypertriglyceridemia as a potential risk factor for the disease is still under debate.2 In fact, results of prospective studies addressing the relation between plasma triglyceride (TG) levels and CHD have failed to show consistent associations.3,4 Hypertriglyceridemia, on the other hand, can be the expression of different lipoprotein abnormalities in the synthesis and catabolism of TG-rich lipoproteins, and the impact of elevated TG levels on CHD may be variable in different patients.5 The presence of concomitant lipoprotein abnormalities, for example, reduced high density lipoprotein (HDL) levels and altered plasma lipoprotein structure, is also important in determining the increased cardiovascular risk in hypertriglyceridemia.5

The fibrinolytic system is regulated by the balance between the levels of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1).6 Alterations of plasma fibrinolytic capacity are known to occur in pathological conditions associated with thrombotic episodes.7,8 Previous data have suggested that reduced fibrinolytic capacity could be associated with the hypertriglyceridemic state.9-11 On the other hand, a correlation between elevated levels of PAI-1 and TG has been found in patients with CHD and in survivors of myocardial infarction,12-14 thus suggesting that elevated levels of PAI-1, in addition to TG, may represent a risk factor in precipitating acute thrombus formation.

To better understand the relation between hypertriglyceridemia and the fibrinolytic system, we have evaluated plasma fibrinolytic capacity, as well as the levels of single components of the fibrinolytic system, in a selected group of patients with a diagnosis of type IV hyperlipidemia and with no history or clinical sign of CHD before and after a dietary treatment aimed at reducing TG levels. The issue of the influence of TG-rich lipoproteins (very low density lipoproteins [VLDLs]) on the release of PAI-1 was also addressed in in vitro experiments with cultured cells.
Patients

Thirty hypertriglyceridemic patients free of other recognized risk factors for atherosclerotic disease, with the exception of cigarette smoking, were recruited from among outpatients attending our Lipid Clinic (E.G. Paoletti Center). The patients were diagnosed with type IV hyperlipoproteinemia according to World Health Organization criteria. None of the patients had a positive history of cardiovascular disturbances or a significant history of alcohol abuse, nor were they taking hormonal therapies or drugs known to interfere with lipid or fibrinolytic parameters. All patients underwent a resting electrocardiogram, and patients with positive electrocardiographic tracings were not considered.

Overt diabetic, hypertensive, and obese patients were not admitted to the study (exclusion criteria: fasting glycemia >110 mg/dl, diastolic blood pressure >95 mm Hg and/or systolic blood pressure >155 mm Hg, and body mass index [BMI] >30 kg/m²). Patient selection was done on the basis of at least two determinations of plasma lipids, which were performed within 2 weeks.

At time zero (basal), plasma lipid and fibrinolytic profiles were determined. Then the patients were instructed to follow a low-carbohydrate diet according to established guidelines in Italy: 40% from fats with a polyunsaturated to saturated fat ratio >1.8, 38–40% from carbohydrates, and 20% from protein. Plasma lipid levels and fibrinolytic parameters were determined in 28 of the 30 patients after 2 months of treatment. Those patients for whom dietary therapy failed to reduce TG levels ≥20% were asked to continue the diet, and at 5 months from the start, all parameters were reevaluated.

Lipids and fibrinolytic values of the patients were compared with those of 27 healthy subjects. The latter subjects were recruited from among the medical staff attending the Clinic and were of similar age and had similar ratios of smokers to nonsmokers and men to women. The subjects were normolipidemic and had dietary habits comparable to those of the patients.

Venous Blood Sampling

Blood samples for evaluation of plasma fibrinolytic activity and measurement of serum lipid parameters were drawn from the antecubital vein of the forearm of fasting subjects (between 9 and 10 AM) after 15 minutes of supine rest. Fibrinolytic activity was also evaluated after venous stasis produced in the contralateral arm by a sphygmomanometer cuff inflated for 10 minutes at a pressure intermediate between the subject's systolic and diastolic arterial pressures. Blood was obtained before deflating the cuff. For fibrinolytic activity, blood was anticoagulated with an equal volume of acetate buffer (pH 3.9) and centrifuged at 12,000g for 60 seconds. The supernatants, divided into small aliquots (150 μl), were further acidified with 10 μl 1N HCl and stored at −70°C. The remaining blood was centrifuged at 800g for 20 minutes at 4°C, and platelet-poor plasma was stored in small aliquots at −70°C until analyzed.

Fibrinolytic Parameters

Euglobulin lysis area (ELA). Plasma fibrinolytic activity was measured in the euglobulin fraction obtained after acidification of diluted plasma (1:10, vol/vol) at pH 5.9 with 0.25% (vol/vol) glacial acetic acid at 4°C. The precipitate was then resuspended in an EDTA–gelatin–barbital buffer (pH 7.8) at the initial volume of plasma, and aliquots of 30 μl each were incubated on the surface of two different fibrin plates for 18 hours at 37°C. Two perpendicular diameters (millimeters) of the lysis produced by euglobulins were then measured in colored plates as previously described.

t-PA activity assay. t-PA activity was measured in plasma, obtained from blood acidified immediately after collection, by a chromogenic method (Ortho Diagnostica System, Milan, Italy). The assay measures the ability of plasma t-PA to convert exogenous plasminogen to plasmin only in the presence of cyanogen bromide fibrin(ogen) fragments and the chromogenic substrate S-2251.

t-PA antigen assay. Plasma levels of t-PA antigen were determined by enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (IMUBIND-5, American Diagnostica, Greenwich, Conn.), following the manufacturer's recommendations. This assay detects both free t-PA and t-PA complexed with inhibitors and includes the use of quenching and irrelevant antibodies to exclude false-positive results. Values are the means of duplicate measurements.

Plasminogen activator inhibitor activity assay. Functional PAI activity was assayed by a two-stage indirect enzymatic assay with reagents obtained from Ortho Diagnostica System. The assay was performed according to the manufacturer's instructions except for minor modifications. t-PA was added to undiluted and diluted (1:2, vol/vol) plasma samples at two different concentrations (40 and 30 IU/ml) and incubated exactly 10 minutes at room temperature, after which the samples were diluted with 2 ml sterile distilled water. The residual t-PA activity was measured by use of the chromogenic substrate S-2251 in the presence of plasminogen and cyanogen bromide fibrin(ogen) fragments.

Plasminogen activator inhibitor antigen assay. Plasma levels of PAI-1 antigen were determined with a commercially available ELISA kit (IMULYSE, Biopool AB, Umeå, Sweden). It includes the use of quenching and irrelevant antibodies to exclude false-positive results. This assay detects both the active and the inactive molecule. Values are the means of duplicate measurements.
Lipid Determinations

Total and lipoprotein cholesterol levels and TG were determined in fresh serum by enzymatic methods. 18,19  HDLs were obtained by selective precipitation with dextran–magnesium chloride; 20  LDLs were prepared by ultracentrifugation according to the National Institutes of Health protocol. 21

Miscellaneous Techniques

Plasma insulin was measured by radioimmunoassay (Sorin Biomedica, Saluggia, Italy), and serum glucose, by enzymatic methods (Carlo Erba Chemicals, Milano, Italy). The levels of β-thromboglobulin in plasma samples were determined by radioimmunoassay (Amersham International, Amersham, UK).

Cell Studies

Cell cultures. Human umbilical vein endothelial cells (ECs) were isolated from individual cords as previously described. 22  Cells were cultured in medium 199 (GIBCO, Mascia Brunelli, Milan, Italy) containing 20% newborn calf serum (GIBCO), 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml heparin (Sigma Chemical Co., St. Louis, Mo.), and 50 μg/ml of a crude preparation of EC growth factor extracted from bovine hypothalamus as described. 23  Cells were subcultured into 75-cm² culture flasks (Costar, Cambridge, Mass.) and allowed to grow until confluent in humidified incubators at 37°C under a 5% CO2/95% air atmosphere. Cells were characterized on the basis of their typical monolayer cobblestone morphology and human factor VIII antigen content (immunofluorescence staining) (Nordic Immunological Lab, Tilburg, The Netherlands). Cells between the second and fourth “in vitro” passages were used. Experiments were performed on cells plated in 24-well (16-mm-diameter) cluster plates and used at confluency.

HepG2 cells, an established cell line derived from a human liver tumor, were grown in Dulbecco’s modified Eagle’s medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin under a 5% CO2/95% air atmosphere. Experiments were performed on cells plated in 24-well cluster plates cultured without changing the medium and were used at confluency.

Lipoprotein isolation. VLDLs were separated by ultracentrifugation from plasma of four normolipidemic donors and of six patients with type IV hypertriglyceridemia. For this purpose, blood was anticoagulated with Na2EDTA (1.4 mg/ml) and centrifuged at 1,000g for 20 minutes to separate plasma. Three milliliters of a potassium bromide solution (d=1.006 g/ml) was placed on 10 ml plasma in cellulose nitrate tubes and centrifuged in a Beckman SW 50 Ti rotor at 40,000 rpm at 4°C for 20 hours. VLDL particles that had floated to the top were then removed, and the total protein content was determined by the Lowry method. 24  Endotoxin contamination of VLDL preparations was determined and consistently found to be below 0.1 ng/mg protein as assessed by the Limulus assay test (Ortho Diagnostica System).

Experimental procedure. Confluent cells were washed three times with phosphate-buffered saline and incubated at 37°C with 1 ml of the appropriate serum-free medium or with VLDL at different total protein concentrations for 15–18 hours. At the end of the incubation, the medium was removed, centrifuged to remove cell debris, and frozen at −20°C after the addition of Triton X-100 (0.25%, vol/vol). Cell supernatant PAI-1 antigen was measured by an ELISA kit (type F1-5, Monozyme, Copenhagen, Denmark), using two different monoclonal antibodies, that allows the detection of free and complexed PAI-1. Appropriate controls were performed to exclude possible interference of VLDL with the assay. Representative cell counts were performed using a Coulter counter (Coulter Electronics Ltd., Luton, England) to ensure that adherent cell number was not affected by the experimental conditions used. In some experiments, the amount of protein remaining on the single wells at the end of the incubation period was determined.

In preliminary experiments, PAI-1 release by ECs and HepG2 cells was shown to increase as a function of incubation time. Levels of PAI-1 in cells incubated with the appropriate medium for a period of 15–18 hours were in the range 9–15 ng/10⁶ cells and 10–30 ng/10⁶ cells for ECs and HepG2 cells, respectively. This incubation time was selected for all experiments aimed at studying the effects of VLDL on PAI-1 release.

Statistical Analysis

Goodness-of-fit χ² was used to test deviation from a normal distribution. Parameters, with the exception of the fibrinolytic, are expressed as mean±SD and were analyzed by unpaired Student’s t test. Group differences in fibrinolytic parameters were analyzed by non-parametric tests (Mann-Whitney U and Wilcoxon), and the data are expressed as medians and ranges. The relation between parameters was assessed by Spearman rank correlation coefficient. For in vitro studies, one-way analysis of variance was used.

Results

Fibrinolytic Parameters in Patients and Controls

Table 1 shows the characteristics of the subjects studied. The two groups were comparable in terms of age, BMI, and plasma glucose and insulin levels. In the hypertriglyceridemics, total cholesterol levels were higher than in controls, whereas LDL cholesterol was comparable in the two groups. HDL cholesterol levels were significantly lower in hypertriglyceridemic patients.

Values of fibrinolytic parameters before and after venous occlusion are shown in Table 2. The basal fibrinolytic activity, as assessed by ELA, was similar in both groups of subjects, and venous occlusion induced a twofold rise in mean fibrinolytic activity.
TABLE 1. Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Male/female (No.)</td>
<td>23/4</td>
<td>26/4</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39±12</td>
<td>45±9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0±2.6</td>
<td>25.3±3.0</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>86.8±8.0</td>
<td>94.7±13.8</td>
</tr>
<tr>
<td>Insulin (µunits/ml)</td>
<td>4.8±3.6</td>
<td>6.5±3.6</td>
</tr>
<tr>
<td>Serum lipids (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.25±0.92</td>
<td>6.67±1.66*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.33±0.25</td>
<td>1.08±0.32*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.37±0.88</td>
<td>3.15±1.55</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>1.05±0.40</td>
<td>5.47±3.29*</td>
</tr>
</tbody>
</table>

Values are mean±SD.
BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.
*p<0.001 for significant differences between hypertriglyceridemic subjects and controls by unpaired Student's t test.

The lack of differences in terms of fibrinolytic activity between groups was supported by the measurements of t-PA activity, the mean increments after venous occlusion being 7.8±6.6 and 7.6±7.9 units/ml in patients and controls, respectively. In contrast, mean values of t-PA antigen, both under resting conditions and after venous occlusion, were significantly higher in hypertriglyceridemics than in controls. Indeed, venous stasis induced a release of 10.9±7.4 and 17.2±10.7 ng/ml t-PA antigen in controls and patients, respectively (p<0.05).

Resting PAI-1 antigen levels in hypertriglyceridemic patients were significantly higher than those of normolipemics. PAI-1 antigen levels in patients were doubled, and this increase paralleled that of PAI activity (Table 2). The concomitantly elevated PAI and t-PA antigen levels may thus account for the lack of differences between the two groups in fibrinolytic response.

When the relation between fibrinolytic parameters and serum lipid profile was considered in the overall population, a significant correlation between PAI antigen and TG levels was found (r=0.38, p<0.001) (Figure 1). PAI-1 antigen levels were also correlated with total cholesterol levels (r=0.40, p<0.001) but not with LDL or HDL cholesterol levels (p>0.05).

In an attempt to understand whether reduction of TG levels could be accompanied by a tendency toward normalization of fibrinolytic parameters, hypertriglyceridemic patients were asked to follow a dietary treatment for 2 months, and 28 of the 30

TABLE 2. Fibrinolytic Parameters Before and After Venous Occlusion in Controls and Hypertriglyceridemic Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n=27)</th>
<th>Patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELA (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>9.5 (5.0-19.7)</td>
<td>9.0 (5.0-16.5)</td>
</tr>
<tr>
<td>After VO</td>
<td>19.0 (5.0-30.5)</td>
<td>16.0 (8.0-29.5)</td>
</tr>
<tr>
<td>t-PA activity (units/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>0.83 (0.17-2.70)</td>
<td>1.00 (0.25-3.16)</td>
</tr>
<tr>
<td>After VO</td>
<td>5.31 (0.36-35.50)</td>
<td>7.58 (1.50-31.01)</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>7.1 (3.2-19.1)</td>
<td>11.4* (4.2-26.6)</td>
</tr>
<tr>
<td>After VO</td>
<td>18.7 (8.3-38.2)</td>
<td>30.2* (13.0-62.8)</td>
</tr>
<tr>
<td>PAI activity (units/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>12.3 (1.2-26.8)</td>
<td>17.5* (0.0-87.0)</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>7.5 (2.5-32.7)</td>
<td>14.7* (3.9-66.0)</td>
</tr>
</tbody>
</table>

Results are expressed as medians, with ranges in parentheses.
ELA, euglobulin lysis area; VO, venous occlusion; t-PA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor.
*p<0.01 for significant differences between hypertriglyceridemic subjects and normal controls by Mann-Whitney U test.

FIGURE 1. Scatterplot showing relation between plasminogen activator inhibitor-1 (PAI-1) antigen (ng/ml) and total serum triglyceride (mmol/l) in the overall population. PAI-1 antigen was correlated positively with the logarithmically transformed serum triglyceride levels. Spearman rank correlation coefficient r=0.38, p<0.001.
TABLE 3. Lipid and Fibrinolytic Parameters of Hypertriglyceridemic Patients Before and After 2 Months of Dietary Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal (n=28)</th>
<th>After diet (n=28)</th>
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</thead>
<tbody>
<tr>
<td>Serum lipids (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.58±1.44</td>
<td>6.20±1.45</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.08±0.33</td>
<td>1.02±0.38</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.15±1.55</td>
<td>3.36±1.32</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>5.27±3.15</td>
<td>4.15±2.30</td>
</tr>
<tr>
<td>Fibrinolytic parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELA (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>8.7 (5.0–16.5)</td>
<td>9.5 (5.0–16.7)</td>
</tr>
<tr>
<td>After VO</td>
<td>15.5 (8.0–29.5)</td>
<td>17.9 (5.7–31.0)</td>
</tr>
<tr>
<td>t-PA activity (units/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>1.00 (0.25–3.16)</td>
<td>0.72 (0.25–2.19)</td>
</tr>
<tr>
<td>After VO</td>
<td>6.24 (1.50–31.01)</td>
<td>6.10 (0.25–32.00)</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>11.8 (4.2–26.6)</td>
<td>12.5 (4.3–20.2)</td>
</tr>
<tr>
<td>After VO</td>
<td>30.2 (13.0–62.8)</td>
<td>25.5 (7.6–56.3)</td>
</tr>
<tr>
<td>PAI activity (units/ml)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before VO</td>
<td>17.5 (0.0–87.0)</td>
<td>15.9 (5.0–70.0)</td>
</tr>
<tr>
<td>After VO</td>
<td>15.8 (3.9–66.0)</td>
<td>13.4 (2.8–49.2)</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/ml)*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Serum lipids are expressed as mean±SD. Fibrinolytic parameters are expressed as medians, with ranges in parentheses.

HDL, high density lipoprotein; LDL, low density lipoprotein; ELA, euglobulin lysis area; t-PA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor.

*Parameters were evaluated before venous occlusion (VO).

Patients agreed with the proposed program. At 2 months from the start of the diet, mean serum TG levels in the overall group of patients were reduced by 22%, but no significant change in the fibrinolytic pattern was observed (Table 3). Also, no effect of the dietary treatment was observed on fasting glucose or insulin levels (data not shown). A positive correlation, however, was found between changes in TG and PAI-1 antigen levels as represented in Figure 2, whereas no significant correlation was observed between changes in TG and t-PA antigen. On the other hand, analysis of the responses of single patients to the diet in terms of TG modifications showed that 14 of the 28 had a TG reduction greater than or equal to 20% (6.19±4.10 and 2.69±1.25 mmol/l mean TG before and after the diet, respectively), with a concomitant decrease of PAI-1 antigen levels of 40%. In fact, in these patients median PAI-1 antigen levels under basal conditions were 22.4 ng/ml (range, 3.9–66) and were 13.4 ng/ml (range, 2.8–49.2) (p<0.05) after the diet. In contrast, in the other 14 patients average TG levels had increased by 28% (4.32±1.23 and 5.53±2.28 mmol/l before and after the diet, respectively), whereas PAI-1 remained unchanged (median PAI-1 antigen, 12.3 ng/ml; range, 5.2–28.3; and median, 13.3 ng/ml; range, 5.2–30, before and after the diet, respectively). This latter group of patients continued the diet for as long as 5 months, but no change in mean TG or fibrinolytic parameters occurred (data not shown).

Effects of Very Low Density Lipoproteins on Plasminogen Activator Inhibitor-1 Release by Endothelial Cells and by HepG2 Cells

For these experiments, VLDLs isolated from single subjects (either normolipidemias or type IV hypertriglyceridemias) were incubated with ECs obtained from single umbilical cords or with HepG2 cells, a hepatoma cell line considered to be functionally representative of human hepatocytes. VLDL isolated from normal donors concentration-dependently stimulated the release of PAI-1 by both cell types, with a significant increment occurring at VLDL concentrations of 25–50 μg/ml protein and with a maximal effect in the range 75–100 μg/ml (Figure 3). That the enhanced levels of PAI-1 found in supernatants of cells (ECs or HepG2 cells)
incubated with VLDL reflected "de novo" synthesis of the protein was confirmed by data indicating that the PAI-1 content of cell extracts was not changed by the presence of VLDL and that PAI-1 levels were no longer increased in cycloheximide-treated (1-10 µg/ml) cells (data not shown). The possible contribution of thrombin bound to VLDL preparations to PAI-1 synthesis by cells was excluded in appropriate experiments with the thrombin inhibitor hirudin (data not shown). VLDL isolated from the plasma of hypertriglyceridemic patients consistently stimulated PAI-1 release by both ECs and HepG2 cells, the effect in HepG2 cells being greater than that in ECs. PAI-1 increased to 19.6±2.7% and 78±8.6% of basal levels in ECs and HepG2 cells, respectively, when challenged with 25 µg/ml VLDL (Figure 4). Neither VLDL preparation (normal or TG-rich) influenced cell (EC or HepG2) morphology or induced cell detachment.

**Discussion**

The association between elevated TG levels and alterations of the fibrinolytic system described in patients with CHD 
12-14,25 has suggested that elevated levels of an inhibitor of the fibrinolytic system, PAI-1, may represent an additional risk factor for atherosclerosis or thrombosis. Indeed, general agreement exists on the direct correlation between PAI and TG levels versus northern European) and/or from the different population studied (Mediterranean or other European) countries in terms of dietary habits and relative risk of CHD.30,31

In the hypertriglyceridemic patients considered in this study, plasma levels of both t-PA antigen, before and after venous occlusion, and resting PAI-1 antigen were significantly higher than those of normolipidemics. However, the increases of these two components of the fibrinolytic system resulted in a net fibrinolytic capacity (t-PA activity) comparable to that of normolipidemics both under resting conditions and after venous occlusion. The finding of normal values of fibrinolytic activity after stimulation is in agreement with data reported by Aznar et al, who found in hypertriglyceridemic CHD patients normal values of total fibrinolytic activity despite an elevated PAI activity. In contrast, others have reported alterations in fibrinolytic activity in hypertriglyceridemia.9,26,32 The apparent discrepancy between our data and those cited above may derive from the different population studied (Mediterranean versus northern European) and/or from the different methods used either to stimulate fibrinolytic activity or to determine fibrinolytic capacity.

Elevated levels of t-PA antigen have been previously reported in CHD patients but, to the best of our knowledge, this is the first report of elevated t-PA levels in patients with primary hypertriglyceridemia. On the other hand, the elevated levels of PAI-1 antigen and activity, as well as the significant correlation between TG and PAI-1 found in type IV patients, are consistent with the results of previous
studies performed of CHD patients with hypertri-
glyceridemia. Moreover, the significant correla-
tion between total, but not LDL, cholesterol levels
and PAI-1 antigen further sustains the role of VLDL
in determining this correlation.

The observation of increased levels of both t-PA
antigen and PAI-1 in hypertriglyceridemics indicates that
in the absence of other recognized risk factors, the
hypertriglyceridemic status, either directly or
indirectly influencing the release of both proteins by
the vascular endothelium, does not impair the phys-
iological capacity to remove generated fibrin and
suggests that, in addition to increases in TG, other
factor(s) are required to impair fibrin removal poten-
tial. This latter concept, however, needs further
investigation in appropriate prospective studies.

It has been suggested35-37 that normalization of
TG levels by dietary means and/or pharmacological
treatment may improve the fibrinolytic pattern. In
considering the overall responses to dietary treat-
ment of our patients, in whom after 2 months of diet
despite an average TG reduction of 22% no change
in fibrinolytic activity or in the single components of
the fibrinolytic system was recorded, we cannot con-
firm this hypothesis. The positive correlation be-
tween TG changes and PAI-1 modifications as the
result of dietary treatment was another strong evi-
dence of a direct linkage between these two param-
ters. Analysis of the single-patient data has shown,
however, that the response of single patients to the
dietary treatment was not homogeneous. According
to Andersen et al.,35 almost 50% of the patients in our
study met the criterion of “good responder” to the
diet (TG reduction >20%; see “Results” section),
and accordingly in this subgroup of patients, PAI-1
antigen levels were significantly reduced. It should,
however, be noted that the two groups of patients
were not comparable in terms of basal values of both
PAI-1 antigen and TG, and thus it cannot be ex-
cluded that the PAI-1 reduction was due to a simple
regression of data toward the mean. Therefore, this
set of data should be considered with caution and
does not permit us to conclude that a causal relation
exists between TG and PAI-1 levels. They provide,
however, the rationale for further prospective studies
aimed at reducing TG levels to within the normal
range, using pharmacological treatments in conjunc-
tion with appropriate dietary procedures. One can
thus envision that the elevated levels of PAI-1 found
in patients with hypertriglyceridemia are a conse-
quense of an interaction of TG-rich lipoproteins with
cells that, in vivo, release PAI-1. Circulating PAI-1
may derive from ECs, hepatocytes, and/or platelet
α-granules,38 and the contribution of each of these
cell types to circulating PAI-1 is unknown. At pre-
cent, we cannot exclude the possibility that the PAI-1
found in the plasma of type IV patients is of platelet
origin, although several studies indicate that platelet
PAI-1 is mostly in inactive form.39 To exclude the
possibility that the PAI-1 measured in our samples
was the result of platelet activation during blood
sampling, β-thromboglobulin levels were measured in
samples with the highest values of PAI-1. In none
of these samples were β-thromboglobulin levels
found to be greater than 1.0 μg/ml.40 It is thus likely
that PAI-1 measured in our series of subjects is the
result of the release of this protein by ECs and/or
liver cells.

It has been recently reported that VLDLs isolated
from normal and hypertriglyceridemic patients con-
sistently stimulate PAI-1 release by ECs,41 and this
has been suggested to provide an explanation for the
clinical finding of an association between serum TGs
and plasma levels of PAI-1. Our data indicate that
VLDLs are capable of increasing PAI-1 release not
only by ECs but also by HepG2 cells, a hepatoma cell
line that has been shown to be representative of
human hepatocytes, thus suggesting for TG-rich lipo-
proteins a broader effect on the behavior of this
inhibitor of the fibrinolytic system. VLDLs isolated
from hypertriglyceridemics were even more effective
on HepG2 cells than on ECs, and this finding can
simply reflect the complex interaction of these lipo-
proteins with hepatic receptors. Indeed, in HepG2
cells at least an apolipoprotein (apo) E receptor
distinct from the classical apo B/E receptor of ECs
has been described.42,43 On the other hand, TG-rich
VLDLs were less potent than normal VLDLs in
increasing PAI-1 release by ECs. As previously men-
tioned, we found that VLDLs from hypertriglyceri-
demics were more effective than normal VLDLs in
stimulating the synthesis and release of PAI-1 by
HepG2 cells, and this difference may have been
underestimated, as suggested by the finding that
larger VLDLs (SI 100-400) are the major factors
responsible for PAI-1 release by cells.41 Indeed, at
equal protein concentration, the number of particles
in TG-rich VLDL is lower than that in normal
VLDL.

Although it is known that the liver plays a funda-
mental role in the synthesis and metabolism of PAI-
1,44 only limited information is available about the
factors influencing these processes. It has been previ-
ously reported that insulin stimulates PAI-1 release
by HepG2 cells.45 The observation that in addition to
insulin, VLDL also stimulates the release of this
protein by liver cells, suggests that these two factors
may potentiate each other in specific conditions such
as combined hyperlipidemia, a condition that appears
to be associated with a high incidence of CHD.

In conclusion, this study shows that in asymptomatic
hypertriglyceridemia, both t-PA and PAI-1 levels are
elevated and that PAI-1 levels tend toward normaliza-
tion in conjunction with TG reduction. In addition, on
the basis of the in vitro data, it can be hypothesized
that the elevated levels of PAI-1 found in plasma of
hypertriglyceridemics are the result of an interaction
of VLDL not only with ECs but also with hepatic cells.
The molecular mechanism(s) responsible for the in-
teraction of these lipoproteins with cells are presently
the object of extensive studies.
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