Levels of Three Hemostatic Factors in Relation to Serum Lipids

Monocyte Procoagulant Activity, Tissue Plasminogen Activator, and Type-1 Plasminogen Activator Inhibitor

David J. Crutchley, Gizelle V. McPhee, Martin F. Terris, and Maria A. Canossa-Terris

To explore the relationship between blood lipid levels and a predisposition to thrombosis, levels of three hemostatic factors were measured in 41 human subjects and correlated with serum lipids. Procoagulant activity associated with peripheral blood monocytes isolated and purified after a 2-hour incubation in whole blood was not significantly related to lipid levels. However, activity in monocytes incubated with 100 ng/ml of bacterial endotoxin was significantly correlated with high density lipoprotein (HDL) cholesterol ($r=0.55$, $p<0.005$), while net procoagulant activity (endotoxin-challenged minus basal) was significantly correlated with both HDL cholesterol ($r=0.61$, $p<0.005$) and total cholesterol ($r=0.50$, $p<0.01$). Plasma levels of the fibrinolytic factor, tissue plasminogen activator, were significantly correlated with total cholesterol ($r=0.41$, $p<0.01$), while those of the type-1 plasminogen activator inhibitor were significantly correlated with both total cholesterol ($r=0.46$, $p<0.01$) and total triglycerides ($r=0.31$, $p<0.05$). The balance between the fibrinolytic factors was not significantly related to serum lipids. These results suggest that the expression of procoagulant activity by peripheral blood monocytes exposed to endotoxin may be enhanced in cases where HDL cholesterol levels are high. In addition, these results suggest that hypertriglyceridemia may be associated with a decreased fibrinolytic capacity due to elevated secretion of plasminogen activator inhibitor. (Arteriosclerosis 9:934–939, November/December 1989)
Informed consent was obtained. A total of 40 ml of blood was drawn by standard forearm venipuncture into sterile, evacuated tubes containing the appropriate anticoagulants: 20 ml was anticoagulated with heparin, 10 ml was anticoagulated with ethylenediaminetetraacetic acid (EDTA), and the remaining 10 ml was used to prepare the serum. All persons were requested to fast overnight, and blood was drawn between 8:30 and 10:00 A.M. to minimize diurnal variations in IPA and PAI-1.7,8

**Monocyte Preparation**

Heparinized blood was immediately divided into eight 2-ml portions. Four portions were mixed with 100 ng/ml endotoxin (lipopolysaccharide W, E coli 011:B4; Difco Labs, Detroit, MI), and the remainder was mixed with an equal volume of sterile saline. The blood was incubated for 2 hours at 37°C with frequent mixing. Mononuclear cells were collected by density gradient centrifugation on Ficoll-Hypaque cushions and were further purified by selective adherence to gelatin-coated dishes. The resulting cells were washed and resuspended in buffer (110 mM NaCl, 50 mM Tris, pH 7.4) before assay of procoagulant activity. Portions were taken for determination of cell count by standard hemocytometric techniques. Cell preparations obtained by these techniques routinely contained more than 95% monocytes, as determined by Wright-Giemsa staining and nonspecific esterase staining and were routinely greater than 90% viable, as determined by trypan blue exclusion.

**Monocyte Procoagulant Activity**

A modification of the method of Bolhuis et al.10 was used. In this method, monocytes activate Factor X in the presence of Ca++, phospholipid, and trace amounts of plasma as a source of Factor VII. The Factor Xa generated is then measured by its ability to cleave a specific chromogenic substrate, N-benzyol-Glu-Gly-Arg-p-nitroanilide (Kabi S-2222; Helena Labs; Beaumont, TX). Assay mixtures contained: 25 μl of a suspension containing approximately 5×10⁶ cells/ml of purified monocytes, plus 65 μl of a mixture consisting of 32 μl of buffer (110 mM NaCl, 50 mM Tris, pH 7.4), 32 μl of 25 mM CaCl₂, 1 μl phospholipid (rabbit brain cephalin, Sigma, St. Louis, MO), 2.5 μl of a solution containing 10 units/ml of purified human Factor X (Sigma), and 0.25 μl of normal human plasma. The mixtures were incubated for 30 minutes at 37°C to allow the formation of Factor Xa. The reaction was stopped by the addition of 45 μl of 10 mM EDTA; then, 100-μl subsamples were transferred into 96-well flat-bottomed plates. An aliquot of 20 μl of a 3 mM solution of the chromogenic substrate was then added, and absorbance at 492 nm was measured. This assay was calibrated by standards traceable to international standards: AU 5061, which was calibrated by standards trace-
Table 1. Clinical Profiles of Human Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55</td>
<td>58</td>
<td>11</td>
<td>26–69</td>
</tr>
<tr>
<td>Serum lipids (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>254</td>
<td>243</td>
<td>64</td>
<td>133–363</td>
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<tr>
<td>HDL cholesterol</td>
<td>65</td>
<td>61</td>
<td>23</td>
<td>32–139</td>
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<tr>
<td>LDL cholesterol</td>
<td>171</td>
<td>166</td>
<td>53</td>
<td>69–294</td>
</tr>
<tr>
<td>LDL cholesterol, calculated</td>
<td>144</td>
<td>143</td>
<td>58</td>
<td>0–263</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>232</td>
<td>165</td>
<td>282</td>
<td>47–1735</td>
</tr>
<tr>
<td>Monocyte procoagulant activity, (units/10⁶ cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67</td>
<td>40</td>
<td>70</td>
<td>0–296</td>
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<tr>
<td>+Endotoxin</td>
<td>163</td>
<td>128</td>
<td>143</td>
<td>0–640</td>
</tr>
<tr>
<td>Net</td>
<td>105</td>
<td>64</td>
<td>135</td>
<td>0–640</td>
</tr>
<tr>
<td>Fibrinolytic factors (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>tPA antigen</td>
<td>19.9</td>
<td>18.7</td>
<td>9.1</td>
<td>5.3–34.7</td>
</tr>
<tr>
<td>PAI-1 antigen</td>
<td>41.0</td>
<td>38.4</td>
<td>17.2</td>
<td>17.4–109.4</td>
</tr>
</tbody>
</table>

There were 15 women and 26 men in the study.

HDL = high density lipoprotein cholesterol, LDL = low density lipoprotein cholesterol, tPA = tissue plasminogen activator, PAI-1 = plasminogen activator inhibitor.

Results

Lipid Profile

A total of 41 persons were studied, 15 women and 26 men, with ages ranging from 26 to 69 years. As shown in Table 1, total serum cholesterol in the persons studied ranged from 133 to 363 mg/dl, and total serum triglycerides ranged from 47 to 1735 mg/dl. Affinity chromatography of thawed serum samples on heparin-agarose columns gave alpha fraction (HDL) cholesterol values in the range of 32 to 139 mg/dl, and beta fraction (LDL) cholesterol values in the range of 69 to 294 mg/dl. When LDL cholesterol was estimated according to the Friedewald equation, values in the range 0 to 263 mg/dl were obtained. The apparent lack of LDL in one patient and the extremely low value (8 mg/dl) observed in another reflect the inaccuracy of this calculation due to the extremely high triglyceride levels in these patients (1735 and 618 mg/dl, respectively).

Monocyte Procoagulant Activity

Monocytes were incubated with whole blood to expose the cells to their native lipid milieu and to approximate in vivo conditions. Monocytes were also incubated in the presence or absence of bacterial endotoxin to assess the effects of lipids on basal and on stimulated activity.

As shown in Table 1, the monocyte-associated procoagulant activity in the persons studied was extremely variable. This has been noted by other workers. Exposure of blood to endotoxin led to an increased procoagulant activity in most persons (30 of 41). In the remainder, procoagulant activity was unchanged (one subject) or declined (nine subjects) after endotoxin treatment. The reasons for the decline are unclear. The monocytes from one person were lost during preparation. The overall stimulation of procoagulant activity in the 40 persons studied was two- to threefold.
Monocyte procoagulant activity was not significantly related to age, gender, LDL cholesterol, or serum triglycerides. However, significant relationships were observed between procoagulant activity and HDL cholesterol. Thus, although basal procoagulant activity was unrelated to HDL cholesterol (Figure 1A), a moderate, positive, and highly significant correlation was found between endotoxin-stimulated activity and HDL cholesterol ($r=0.55, p<0.005$; Figure 1B). A stronger and equally significant correlation was found between the net increase in procoagulant activity (i.e., endotoxin-stimulated minus basal) and HDL cholesterol ($r=0.61, p<0.005$; Figure 1C). An additional correlation was found between the net increase in procoagulant activity and total cholesterol ($r=0.60, p<0.01$), although multivariate analysis indicated that total cholesterol was not an independent variable.

**Fibrinolytic Factors**

Table 1 shows that plasma tPA antigen levels ranged from 5.3 to 34.7 ng/ml, and plasma PAI-1 antigen levels ranged from 17.4 to 109.4 ng/ml. Normal values for plasma tPA antigen have generally been reported to be in the range of 1 to 13 ng/ml, while those for PAI-1 antigen are in the range of 4 to 77 ng/ml. Since the antibodies used in the ELISA for tPA recognize both free and inhibitor-bound tPA while those used in the ELISA for PAI-1 recognize only free PAI-1, these data indicate that in most cases an excess of PAI-1 is present. Similar observations have been made by others and are consistent with the finding that most of the tPA in plasma is catalytically inactive.

An analysis of the relationship between the levels of the fibrinolytic factors and serum lipids showed weak but significant correlations. Thus, tPA was positively correlated with total cholesterol ($r=0.41, p<0.01$; Figure 2A) and with cholesterol associated with the unadjusted beta (LDL) fraction ($r=0.39, p<0.01$), although multivariate analysis indicated that the latter was not an independent variable. Moreover, when the values for calculated LDL cholesterol were used, the correlation was lost ($r=0.23, p>0.05$). Finally, a moderate but significant correlation was observed between PAI-1 and total cholesterol ($r=0.46, p<0.01$; Figure 2B), and a weak correlation was found between PAI-1 and total triglycerides ($r=0.31, p<0.05$; Figure 3). No correlation was found between PAI-1 and...
it is noteworthy that exposure of isolated human contrast, however, Carson has reported that HDL and development of procoagulant activity via a prothrombinase complex. In this context, it is noteworthy that exposure of isolated human peripheral blood monocytes to purified HDL enhanced the development of procoagulant activity via a prothrombinase, which was later shown to be Factor VII. In contrast, however, Carson has reported that HDL and apoprotein A-II inhibit the activation of Factor Xa by purified tissue factor and Factor VII. Whatever the mechanisms involved, the apparent positive relationship between HDL cholesterol and the development of monocyte procoagulant activity requires independent confirmation, in view of the fact that HDL, a negative risk factor for coronary heart disease, is generally regarded as a "good" cholesterol. Studies identifying the subclass of HDL most closely associated with increased monocyte procoagulant activity would also seem to be warranted.

The present results also indicate weak but significant correlations between HDL cholesterol and fibrinolytic factors, t-PA and PAI-1. In the case of t-PA, the association appeared to be with the LDL fraction. However, the clinical effects of hypercholesterolemia on the fibrinolytic system are difficult to predict, since both factors increased in concert. Thus, no significant changes were seen in the balance between t-PA and PAI-1 with respect to cholesterol or LDL levels. In contrast, a weak but significant correlation was found between PAI-1, but not t-PA, and serum triglyceride levels. These results confirm the findings of others and support the suggestion that hypertriglyceridemia may be associated with an increased tendency to thrombosis due to impaired fibrinolytic capacity.

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**References**


Index Terms: blood coagulation • fibrinolysis • monocyte procoagulant activity • tissue factor • tissue plasminogen activator • plasminogen activator inhibitor • serum cholesterol • serum triglycerides • hyperlipidemia
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