Plasma Lipoproteins Enhance Tissue Factor–Independent Factor VII Activation

Marianne Kjalke, Angela Silveira, Anders Hamsten, Ulla Hedner, Mirella Ezban

Abstract—The effect of plasma lipoprotein fractions (large very-low-density lipoprotein, small very-low-density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein) on initiation of blood coagulation by supporting factor VII activation or by stimulating monocytes to express tissue factor was investigated in vitro. Endotoxin-free preparations of lipoprotein fractions did not induce functional tissue factor in monocytes, whereas all lipoprotein fractions enhanced tissue factor–independent activation of factor VII by factor Xa and by factors Xa/Va. In contrast, no or only slight enhancement of factor IXa–, factor IXa/VIIa–, factor XIa–, or thrombin-mediated factor VII activation was observed. The effect of small very-low-density lipoprotein was less than that of large very-low-density lipoprotein, and intermediate-density and low-density lipoproteins caused an even lower but still significant increase of factor Xa– and factor Xa/Va–mediated factor VII activation. When the data were normalized for apolipoprotein B-100 content, differences remained between lipoprotein fractions. In contrast, when phospholipid content was used for normalization, differences between lipoprotein fractions in factor Xa– and factor Xa/Va–mediated factor VII activation disappeared, indicating that phospholipids were involved in factor VII activation. This was supported by enhancement of factor Xa–mediated factor VII activation by synthetic phospholipid vesicles containing negatively charged phospholipids. (Arterioscler Thromb Vasc Biol. 2000;20:1835-1841.)

Key Words: coagulation ■ factor VII ■ lipoproteins ■ monocytes ■ tissue factor

Activated coagulation factor VII (factor VIIa) in complex with its cofactor and receptor tissue factor (TF) is believed to be the principal activator of blood coagulation in vivo. Under normal circumstances, functional TF is expressed only on cells separated from the circulation. After vascular injury, TF will be exposed to plasma proteins and bind factor VIIa, dramatically enhancing its procoagulant activity. In arteriosclerotic lesions, TF expression is seen on endothelial cells, macrophages, and smooth muscle cells. Activation of factor VII by factor VIIa in which both zymogen and enzyme are bound to a TF molecule. Intrinsic pathway activation of factor VII, either directly by factor XIIa or through factor XIIa–mediated activation of factor XI and subsequent factor IX activation, has been observed in vitro, but the importance of these mechanisms in vivo remains unclear.

During alimentary lipemia, plasma concentrations of factor VIIa and factor IX activation peptide are increased, whereas the level of factor XIIa remains constant. Furthermore, postprandial activation of factor VII was not observed in 2 patients with factor XI deficiency or in 3 patients with factor IX deficiency but did occur in 2 patients with factor XII deficiency. In contrast, Miller et al observed activation of factor VII in healthy adults and in factor XII– and factor XI–deficient patients after a high-fat meal but not in factor IX–deficient patients. These in vivo data indicate that factor IX is involved in postprandial activation of factor VII, whereas involvement of factor XI is debatable. Factor XIIa, on the other hand, seems not to be involved.

Plasma lipoproteins, in particular very low density lipoprotein (VLDL), have been shown to support prothrombinase activity in vitro both in the presence of factors Xa and Va and when the reaction was initiated with TF and factor VIIa. In vivo a positive correlation between plasma level of factor VIIa and large triglyceride-rich lipoprotein particles (chylomicrons and VLDL) has been found in non-

Received July 5, 1999; revision accepted December 15, 1999.
From Tissue Factor/Factor VII Research (M.K., U.H., M.E.), Novo Nordisk, Måløv, Denmark; and the Atherosclerosis Research Unit (A.S., A.H.), King Gustaf V Research Institute, Karolinska Institutet, Karolinska Hospital, Stockholm, Sweden.
Correspondence to Marianne Kjalke, PhD, Tissue Factor/Factor VII Research, Novo Nordisk A/S, C9.1.29, Novo Nordisk Park, DK-2760 Måløv, Denmark. E-mail mkja@novo.dk
© 2000 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

1835
TABLE 1. Characterization of Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Lipoprotein Type</th>
<th>ApoB-100 (μg/mg protein)</th>
<th>TFPI (U/mg protein)</th>
<th>Phospholipid (μmol/mg protein)</th>
<th>Phospholipid per Particle* (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting lipoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large VLDL</td>
<td>0.05±0.02</td>
<td>0.08±0.00†</td>
<td>1.9±0.4</td>
<td>20 545±3934</td>
</tr>
<tr>
<td>Small VLDL</td>
<td>0.10±0.01</td>
<td>0.28±0.05§</td>
<td>1.7±0.4</td>
<td>9676±3448</td>
</tr>
<tr>
<td>IDL</td>
<td>0.6±0.1</td>
<td>0.5±0.2</td>
<td>1.2±0.2</td>
<td>1167±343</td>
</tr>
<tr>
<td>LDL</td>
<td>1.2±0.4</td>
<td>1.0±0.3</td>
<td>1.1±0.1</td>
<td>574±298</td>
</tr>
<tr>
<td>Postprandial lipoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large VLDL</td>
<td>0.06±0.02</td>
<td>0.08±0.02†</td>
<td>2.2±0.3</td>
<td>20 104±4929</td>
</tr>
<tr>
<td>Small VLDL</td>
<td>0.08±0.03</td>
<td>0.25±0.05§</td>
<td>1.7±0.3</td>
<td>13 285±4991</td>
</tr>
<tr>
<td>IDL</td>
<td>0.8±0.1</td>
<td>0.6±0.4</td>
<td>1.2±0.2</td>
<td>888±191</td>
</tr>
<tr>
<td>LDL</td>
<td>1.3±0.3</td>
<td>0.9±0.4</td>
<td>1.1±0.1</td>
<td>480±97</td>
</tr>
</tbody>
</table>

Values are mean±SD of lipoprotein fractions prepared from plasma for 5 donors.
*Phospholipid per particle was calculated as the molar ratio of phospholipid to apoB-100 because each lipoprotein particle contains 1 apoB-100 molecule. A molecular mass of 549 000 was used for apoB-100.
†TFPI content was below the detection limit in large VLDL fractions from 3 donors (ie, values are mean±SD of 2 samples).
‡In small VLDL from 1 donor, TFPI content was below the detection limit (ie, values are mean±SD of 4 samples).

Isolation and Characterization of Lipoprotein Fractions

Blood samples were obtained from fasting healthy donors before and 3 hours after a standard mixed meal. Blood was drawn into precooled EDTA-containing tubes (Vacutainer, Becton Dickinson), which were immediately placed in an ice-water bath. Plasma was recovered within 30 minutes by low-speed centrifugation (20 minutes at 1750g) at 1°C and kept at this temperature throughout the preparation procedures. Phospholipids (Sigma; final concentration 10 mmol/L) and aproatin (Trasylo; Bayer; final concentration 28 mmol/L) were immediately added to the isolated plasma samples before fractionation of triglyceride-rich lipoproteins. The lipoprotein fractions designated as large VLDL (Svedberg flotation rate [Sf] 60 to 400), small VLDL (Sf 20 to 60), intermediate density lipoproteins (IDL, Sf 12 to 20), and low density lipoproteins (LDL, Sf 0 to 12) were isolated by cumulative rate ultracentrifugation as described.

Immediately before use, lipoprotein fractions were transferred into Macrophage SFM media (Life Technologies) or HBS/BSA/CaCl₂ (HBS with 1 mg/mL BSA and 5 mmol/L CaCl₂) by gel filtration on NAP columns (Pharmacia).

Lipoprotein fractions were analyzed for content of total phospholipids (Wako Chemicals GmbH), apoB-100, and TFPI (Table 1). Endotoxin contamination was evaluated in a Limulus amoebocyte lysate chromogenic assay (Kinetic QCL, BioWhittaker); 1 EU/mL corresponded to 0.1 ng/mL Escherichia coli O55:B55 endotoxin. TF antigen was analyzed by ELISA (Imubind TF ELISA kit, American Diagnostica) as recommended in the manufacturer’s instructions. Functional TF was measured in a factor Xa generation assay as described below.

Methods

Subjects

Healthy 50-year-old men participating in a population survey were recruited as donors for lipoprotein isolation. The protocol involving human volunteers was approved by the ethics committee of Karolinska Hospital. All subjects gave informed consent.

Materials

Human coagulation factors IXaβ, X, Xa, and Xla were from Enzyme Research Laboratories; human factor Va was from American Diagnostic; and human α-thrombin was from Boehringer Mannheim. Extracellular domains of TF (residual 1 to 219 [TF1-219]) were prepared from Novo Nordisk. Factor VII was prepared using the same protocol used for factor VIIa except that benzamidine (Sigma Chemical Co) was added to a final concentration of 50 mmol/L throughout the purification procedure. Factor VIII was activated by adding 0.14 μmol/L thrombin and incubating for 2 minutes at 37°C, followed by inhibition of the thrombin by addition of a 10-fold molar excess of H-D-Phe-Pro-Arg-chloromethyl ketone (Bachem) and removal of residual H-D-Phe-Pro-Arg-chloromethyl ketone by gel filtration on a 0.4-ml Sephadex G-25 Fine column (Pharmacia) equilibrated with HEPES-buffered saline (HBS; 20 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl) containing 5 mmol/L CaCl₂, 10% glycerol, and 0.02% Tween-80. The concentration of activated factor VIII (VIIa) was determined with use of the Coatest (Chromogenex) according to the manufacturer’s instructions. Polyclonal goat antibodies against TFPI and TF were made at Novo Nordisk using standard protocols.

Synthetic phospholipids were made by mixing pure phosphatidyl choline (PC); PC (75%) and phosphatidyl serine (PS; 25%); or PC (46%), PS (5%), sphingomyelin (12%), phosphatidyl ethanolamine (12%), phosphatidyl inositol (11%), and lysophosphatidyl choline (14%) in chloroform. All phospholipids were from Sigma. Chloroform was evaporated, and phospholipids were dissolved in HBS containing 50 mmol/L n-octyl β-D-glucopyranoside. For PC vesicles, a higher concentration of detergent was required. Detergent was removed by extensive dialysis against HBS.

Stimulation of Monocytes

Peripheral blood was drawn into heparin-containing tubes (Vacutainer) from healthy donors who had not taken acetylsalicylic acid within the last 10 days or other nonsteroidal antiinflammatory drugs.
with the last 48 hours. Mononuclear cells were isolated using Lymphoprep density-gradient medium (Nycomed, Life Technologies) according to the manufacturer’s instructions, washed with versene buffer (Life Technologies), and resuspended in Macrophage SFM medium. Samples of 5 x 10^7 mononuclear cells in volumes of 200 μL containing ~5% monocytes as determined on a Cell泰q instrument (ILS Laboratories Scandinavia) were incubated with the lipoprotein fractions at a concentration of 50 μg total protein/mL for 20 hours at 37°C in 5% CO₂.

Assay for Functional TF
Expression of TF function on intact monocytes in suspension and directly on the lipoprotein fractions was determined in a factor Xa generation assay. Monocytes were washed twice in HBS/BSA/CaCl₂, samples were divided into 2 aliquots, and 1 aliquot of each sample was incubated for 15 minutes with 0.2 mg/mL anti-TF immunoglobulin (IgG). Factor VIIa was added to a final concentration of 10 nmol/L, and after 30 minutes factor X was added to a final concentration of 135 nmol/L. The reaction was stopped after 30 minutes at 37°C by addition of an equal volume of HBS containing 1 mg/mL BSA and 10 nmol/L EDTA. Chromozyme X (Boehringer Mannheim) was added to a concentration of 0.5 mmol/L, and absorbance at 405 nm was measured continuously for 20 minutes on a Spectramax 340 plate reader (Molecular Devices). Absorbance values (ΔA405/min) were converted to factor Xa concentrations by using a standard curve of factor Xa activity.

Factor VII Activation
Lipoprotein fractions (final concentration 50 μg total protein/mL) or phospholipid vesicles (final concentration 0.1 to 100 μmol/L) in HBS/BSA/CaCl₂ were incubated for 15 minutes with 10 μg/mL anti-TFPI IgG. This concentration of anti-TFPI IgG was >10-fold higher than required for neutralizing all TFPI activity in LDL fractions. Factor VII was added to a concentration of 10 nmol/L, and either factor Xla, Xa, Xa/Va, IXa, IXa/VIIIa, or thrombin were added to a concentration of 1 nmol/L, and the samples incubated at 37°C for 1 hour. The reaction was stopped by addition of EDTA to a concentration of 6 mmol/L. The factor VIIa formed was measured in a factor VIIa–specific clotting assay using TF1–219 as previously described except that samples or factor VIIa standard dilutions were mixed with an equal volume of factor VII–deficient plasma (Helena Laboratories) and that 80 nmol/L TF1–219 was mixed with 15 nmol/L CaCl₂, in one reagent reservoir and rabbit brain cephalin (Hemachem Inc) diluted 20-fold in Tris-buffered saline (TBS)/BSA (50 mmol/L Tris-Cl, pH 7.4, containing 100 mmol/L NaCl and 1 mg/mL BSA) was placed in another reagent reservoir in the ACL 300 coagulometer (ILS Laboratories). Clotting times were converted to factor VIIa concentrations by comparison with a standard curve of factor VIIa. Neither factor Xla, Xa, Xa/Va, IXa, or IXa/VIIIa nor thrombin alone at the concentrations used in the assay (without factor VII) influenced the measurements. The presence of phospholipid vesicles did not shorten the clotting time of factor VIIa.

Factor IXa/VIIIa–Mediated Factor X Activation
Large VLDL, small VLDL, LDL (final concentration 50 μg/mL), or buffer (HBS/BSA/CaCl₂) was incubated with anti-TFPI IgG as described above. Factors IXa and VIIIa were added to final concentrations of 1 nmol/L, and factor X was added to a concentration of 135 nmol/L. The reaction was stopped after 1 minute of incubation at 37°C by adding excess EDTA. The factor Xa formed was determined as described above for the functional TF assay.

Control of Presence of Platelets or Microparticles in Lipoprotein Fractions
Microparticles were prepared from platelets isolated from peripheral blood to be used as a positive control. Mononuclear cells were isolated on Lymphoprep density-gradient centrifugation medium as described above, and platelets were isolated as described. Microparticles were generated by incubating the platelets with 10 μmol/L calcium ionophore A23187 (Calbiochem) for 15 minutes at 37°C. Lipoprotein fractions (volumes between 0.6 and 1.3 mL containing lipoproteins at concentrations of 40 to 1480 μg/mL), a similar volume (1.0 to 1.5 mL) of buffer, unactivated platelets, and the microparticle preparation were centrifuged for 20 minutes at 25 000g, and the supernatants were removed. Pellets were resuspended in 100 μL HBS/BSA containing 0.1% NaN₃, divided into 2 aliquots, and labeled with either FITC-conjugated monoclonal antibody to CD41 (GPIIIa, αIIb integrin, Pharmingen, CD416) and peridinin chlorophyll protein (PerCP)-conjugated monoclonal antibody to CD61 (GPIIIa, integrin β₃, Becton Dickinson) or FITC- and PerCP-conjugated mouse IgG1, control antibodies (Becton Dickinson). Filtered 2% paraformaldehyde in HBS was added to a final volume of 0.5 mL before samples were analyzed for CD41- and CD61-positive particles on a FACScan flow cytometer (Becton Dickinson). Forward- and side-angle light scatter and fluorescence channels were set on log scale. The threshold was set at 25 on the forward-angle light scatter. A gate including both unactivated platelets and ionophore A23187–activated platelets and microparticles was set on a FITC versus PerCP fluorescence dot plot, and events within this gate were defined as particles of potential platelet origin. These CD41- and CD61-positive particles were quantified by adding 400-μL samples to TruCount tubes (Becton Dickinson) and measuring the number of CD41- and CD61-positive particles relative to the number of fluorescent beads in the tubes as described in the manufacturer’s instructions. Antibodies diluted in buffer were used as a control of background fluorescence.

Results
Factor VII Activation
Lipoprotein fractions (large VLDL, small VLDL, IDL, and LDL) were tested for associated TF, which could, if present, in complex with plasma factor VIIa activate zymogen factor VII, increasing the level of plasma factor VIIa. No TF antigen could be determined in an ELISA (data not shown). The TF activity of lipoprotein fractions was around background level and not statistically different from that of samples preincubated with a blocking antibody to TF. This shows that the lipoprotein fractions did not contain TF.

To evaluate whether lipoproteins can provide TF-independent factor VII activation, zymogen factor VII was incubated with various enzymes, with or without their activated cofactors, in the presence and absence of lipoprotein fractions, and the factor VIIa formed was measured in a factor VIIa–specific clotting assay (Figure 1a). Lipoproteins alone did not cause factor VII activation. In the presence of factors Xa or Xa/Va, all lipoprotein fractions caused a significant increase in factor VII activation. In contrast, only large VLDL caused a significant increase in factor IXa– or factor IXa/VIIIa–mediated factor VII activation, but the effect was much less pronounced than with factor Xa or Xa/Va. Factor Xla did not activate factor VII, and the lipoprotein fractions did not enhance thrombin-mediated factor VII activation (data not shown). Large VLDL was the most potent lipoprotein fraction enhancing factor Xa– and factor Xa/Va–mediated factor VII activation; ie, a significant difference was observed between large VLDL and small VLDL, IDL, and LDL. Likewise, small VLDL had a greater effect than either IDL or LDL, whereas no difference was observed between IDL and LDL. No differences were observed between fasting and postprandial lipoproteins when fixed concentrations of lipoproteins were used (data not shown).

Lipoprotein particles consist of a hydrophobic core containing triglycerides and esterified cholesterol surrounded by an outer coat comprising phospholipids, free cholesterol, and apolipoproteins. One molecule of apoB-100 is present on each large and small VLDL, IDL, and LDL particle. To
determine whether the phospholipid or apoB-100 components of lipoprotein particles were responsible for promoting factor VII activation, lipoprotein fractions were analyzed for their phospholipid and apoB-100 content (Table 1) and factor VII activation data were normalized using these values (Figure 1b and 1c). After normalization for apoB-100 content, differences between lipoprotein fractions in enhancing factor Xa– and factor Xa/Va–mediated factor VII activation persisted. This indicates that apoB-100 per se was not responsible for the observed enhancement. When the factor VII activation data were normalized to phospholipid content (Figure 1c), differences in enhancement of factor Xa–mediated factor VII activation observed between the lipoprotein fractions disappeared.

To further examine whether the phospholipid content of the lipoprotein fractions could be responsible for the observed enhancement of factor Xa– and factor Xa/Va–mediated factor VII activation, the ability of synthetic phospholipid vesicles to enhance factor Xa–mediated factor VII activation was evaluated. Vesicles containing pure PC, PC (75%) and PS (25%; PCPS vesicles) or vesicles with the same phospholipid composition as reported for lipoprotein fractions (46% PC, 12% sphingomyelin, 5% PS, 12% phosphatidyl ethanolamine, 11% phosphatidyl inositol, and 14% lysophosphatidyl choline) were prepared and used in the factor VII activation assay (Figure 2). Phospholipid vesicles containing negatively charged phospholipids (PCPS and vesicles with a composition similar to that of phospholipids in lipoprotein particles) enhanced factor Xa–mediated factor VII activation in a dose-dependent manner, but PC vesicles had no effect.

Tenase Activity
In vivo data suggest the involvement of factor IX in postprandial activation of factor VII. Because lipoprotein fractions had only a minor effect on factor IXa–mediated factor VII activation (Figure 1), involvement of factor IX could be through generation of factor Xa, which in turn could activate factor VII. Therefore, the ability of lipoprotein fractions to support tenase activity was analyzed (Figure 3). Large VLDL enhanced factor X activation most efficiently, whereas small VLDL had only a modest effect. LDL had only a modest effect on tenase activity. Normalization of the data for phospholipid content did not abolish differences between individual lipoprotein fractions, nor did normalization for apoB-100 content (data not shown). This indicates that components other than phospholipids were involved in supporting tenase activity.

Control of Material of Platelet Origin in Lipoprotein Fractions
Microparticles formed by shedding of membrane vesicles from (activated) platelets are a well-suited surface for assem-
Lipoproteins Enhance Factor VII Activation

Kjalke et al

Discussion

In this study, we showed that plasma lipoproteins, particularly the larger triglyceride-rich lipoproteins, promoted initiation of coagulation by enhancing factor Xa– and factor Xa/Va–mediated factor VII activation (Figure 1). In contrast, lipoprotein fractions had no or a negligible effect on factor IXa– and factor IXa/VIIa–mediated factor VII activation. The possibility of TF-independent activation of factor VII by factor Xa or Xa/Va is supported by the observation of Butenas and Mann11 that TF does not increase the rate of

Figure 3. Tenase activity in the presence of lipoprotein fractions. Lipoprotein fractions (50 μg/mL total protein) or buffer were mixed with factors IXa and VIIa (both at a concentration of 1 nmol/L) and factor X (135 nmol/L). After 1 minute of incubation at 37°C, the reaction was stopped and factor Xa was measured using a chromogenic substrate. Values are mean and SEM of duplicate analyses of lipoprotein fractions from 4 donors (large VLDL) or 2 donors (small VLDL and LDL).

Table 2. Functional TF Expression on Monocytes After Incubation With Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>TF (nmol/L Xa · min⁻¹ · 10⁻⁶ monocytes)</th>
<th>Endotoxin (EU/mL)</th>
<th>Determined.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
<td>Donor 2</td>
<td>Donor 3</td>
</tr>
<tr>
<td>Starting lipoproteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large VLDL</td>
<td>13±2</td>
<td>&lt;0.004</td>
<td>2±0.1</td>
</tr>
<tr>
<td>Small VLDL</td>
<td>10±2</td>
<td>&lt;0.006</td>
<td>4±2</td>
</tr>
<tr>
<td>IDL</td>
<td>12±4</td>
<td>&lt;0.36</td>
<td>48±1</td>
</tr>
<tr>
<td>LDL</td>
<td>(272±1311)†</td>
<td>0.11</td>
<td>4±3</td>
</tr>
<tr>
<td>Postprandial lipoproteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large VLDL</td>
<td>12±3</td>
<td>&lt;0.003</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Small VLDL</td>
<td>11±4</td>
<td>&lt;0.008</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>IDL</td>
<td>(225±76)†</td>
<td>1.7</td>
<td>2±1</td>
</tr>
<tr>
<td>LDL</td>
<td>(212±82)†</td>
<td>7.55</td>
<td>4±2</td>
</tr>
</tbody>
</table>

Monocytes were isolated from peripheral blood and incubated with or without 50 μg/mL lipoproteins for 20 h, and TF expression was measured in a factor Xa generation assay. Values are mean ± SD of 3 samples. Lipoprotein fractions were tested for endotoxin in a Limulus amoebocyte lysate assay. ND indicates not determined.

* A 2-tailed paired t test was used to compare TF expression on monocytes incubated with lipoprotein fractions with TF expression on monocytes incubated with no additives.

†Values omitted from statistical comparisons because of detectable endotoxin content.
factor VII activation by factor Xa. The increased plasma level of factor VIIa seen after a high-fat meal in normal individuals, but not in factor IX–deficient patients, indicates that factor IX is involved in the lipoprotein-supported activation of factor VII. This could then occur through activation of factor X to factor Xa, which in turn could activate factor VII on the surface of the lipoprotein particles. Indeed, the larger lipoprotein particles were able to enhance factor Xa generation by factor IXa/VIIa (Figure 2).

The components of lipoprotein particles responsible for the observed enhancement of factor Xa–mediated factor VII activation are most likely the phospholipids, as indicated by the disappearance of differences between lipoprotein fractions in ability to support factor VII activation when the activities were normalized to phospholipid content of the lipoprotein fractions. This is supported by the association between factor VIIa and serum phospholipid concentrations in a cohort study. In addition, synthetic phospholipid vesicles containing negatively charged phospholipids were able to increase factor Xa–mediated factor VII activation (Figure 2). Rota et al. and Moyer et al. have shown that VLDL and to a lesser extent LDL support prothrombinase activity. In contrast to our results on factor VII activation, the difference between VLDL and LDL in supporting prothrombinase activity remained after the activity was normalized to phospholipid content. When we normalized the factor X activation data to phospholipid content, differences between large VLDL, small VLDL, and LDL persisted. This indicates that the ability of lipoproteins to support factor VII activation depends mainly on phospholipid content, whereas other components are involved in supporting prothrombinase and tenase activity. The content of individual phospholipids (46% PC, 12% sphingomyelin, 5% PS, 12% phosphatidyl ethanolamine, 11% phosphatidyl inositol, and 14% lysophosphatidyl choline) is similar for VLDL, IDL, and LDL, excluding the possibility of a different content of a particular phospholipid as a cause of the observed difference between VLDL and LDL. A lower TFPI content in VLDL than in LDL could be a reason for the higher capacity of VLDL compared with LDL to support prothrombinase activity. However, Moyer et al. have shown that addition of anti-TFPI IgG, which inhibits TFPI in assays comprising phospholipid vesicles, did not alter the ability of LDL to support prothrombinase activity. In our experiments, TFPI was neutralized by preincubating the lipoprotein fractions with anti-TFPI IgG.

In our study, isolated endotoxin-free lipoproteins failed to stimulate monocytes to express functional TF. This is in agreement with the recent data of van den Eijnden et al. showing that native VLDL and LDL did not induce functional TF on human monocyte–derived macrophages. However, this finding contradicts data from other groups showing TF activity on human monocytes or monocyte-derived macrophages after incubation with native VLDL or LDL. In some of these reports, the cells are adhered to tissue culture plates, which we have found can be sufficient to induce TF activity. Also, endotoxin contamination below detection limits may induce TF activity. Brand et al. demonstrated that preparations of oxidized LDL made without special precautions to prevent endotoxin contamination caused TF induction on monocytes, whereas oxidized LDL prepared under endotoxin-free conditions was unable to induce functional TF. Lipopolysaccharide at a concentration as low as 10 pg/mL in the presence of serum can induce TF activity on adherent monocytes. Interestingly, Penn et al. have shown that LDL induces TF mRNA and protein on vascular smooth muscle cells but not TF activity. The latter could be induced with H2O2, which alone did not stimulate the cells to TF synthesis. It is possible that nonfunctional TF could also be induced in monocytes after incubation with lipoproteins and that a certain stimulus is necessary for transforming this TF into a functional cofactor for factor VIIa.

Acknowledgments

This study was supported by a grant from Novo Nordisk A/S to Dr. Angela Silvera. Dr. Lars Christian Petersen is thanked for inspiring discussions. The authors are grateful to Karin Danell-Towerud for assistance with isolation of plasma lipoproteins and to Lone Odborg and Lone Langhoff for technical assistance. Henrik Strandgaard is thanked for the Limulus amoebocyte lysate analysis.

References

17. Miller GJ, Martin JC, Mitropoulos KA, Enouf MP, Cooper JA, Morrissey
JH, Howarth DJ, Tuddenham EGD. Activation of factor VII during ali-
mentary lipemia occurs in healthy adults and patients with congenital factor
XI or factor XI deficiency, but not in patients with factor IX deficiency.
BLOOD. 1996;87:4187–4196.
18. Rota S, McWilliam NA, Baglin TP, Byrne CD. Atherogenic lipoproteins
support assembly of the prothrombinase complex and thrombin gen-
eration: modulation by oxidation and vitamin E. Blood. 1998;91:
508–515.
19. Moyer MP, Tracy RP, Tracy PB, van’t Veer C, Sparks CE, Mann KG.
Plasma lipoproteins support prothrombinase and other procoagulant
20. Mitropoulos KA, Miller GJ, Reeves BEA, Wilkes HC, Cruickshank JK.
Factor VII coagulant activity is strongly associated with the plasma
concentration of large lipoprotein particles in middle-aged men. Athero-
21. Xu N, Dahlbäck B, Ohlin A-K, Nilsson A. Association of vitamin K–de-
pendent coagulation proteins and C4b binding protein with triglycer-
22. Schwartz BS, Levy GA, Curtis LK, Fair DS, Edgington TS. Plasma lipoprotein induction and suppression of the cellular pro-
Enhanced procoagulatory activity (PCA) of human monocytes/macro-
phages after in vitro stimulation with chemically modified LDL. Athero-
24. Wada H, Kaneko T, Wakita Y, Minamikawa K, Nagaya S, Tamaki S,
Deguchi K, Shirakawa S. Effect of lipoproteins on tissue factor activity.
25. Lewis JC, Bennett-Cain AL, DeMars CS, Doellgast GJ, Grant KW, Jones
NL, Gupta M. Procoagulant activity after exposure of monocyte-derived
macrophages to minimally oxidized low density lipoprotein: co-
localization of tissue factor antigen and nascent fibrin fibers at the cell
26. van den Eijnden MMED, van Noort JT, Hollaar L, van der Laarse A,
Bertina RM. Cholesterol or triglyceride loading of human monocyte–
derived macrophages by incubation with modified lipoproteins does not
induce tissue factor expression. Arterioscler Thromb Vasc Biol. 1999;19:
384–392.
pathway inhibitor is expressed by human monocyte–derived macro-
phages: relationship to tissue factor induction by cholesterol and oxidized
28. Freskgård P-O, Olsen OH, Persson E. Structural changes in factor VIIa
induced by Ca2+ and tissue factor studied using circular dichroism spec-
29. Pedersen AH, Nordfang O, Norris F, Wiberg FC, Christensen PM,
Moeller KB, Meidahl-Pedersen J, Beck TC, Norris K, Hedner U, Kissel
W. Recombinant human extrinsic pathway inhibitor: production, iso-
lation, and characterization of its inhibitory activity on tissue factor-ini-
30. Thim L, Bjørn S, Christensen M, Nicolaisen EM, Lund-Hansen T,
Pedersen AH, Hedner U. Amino acid sequence and posttranslational
modifications of human factor VIIa from plasma and transfected baby
acid residues 721–729 are required for full factor VIII activity. Eur J
Hamsten A. Effects of a selective β-blocker on postprandial tri-
glyceride-rich lipoproteins, low density lipoprotein particle size and
glucose-insulin homeostasis in middle-aged men with modestly increased
Activation of coagulation factor VII during alimentary lipemia. Arte-
34. Lowry OH, Roseborough NJ, Fazz AL, Randall RJ. Protein measurement
35. Karpe F, Hamsten A. Determination of apolipoproteins B-48 and B-100
in triglyceride-rich lipoproteins by analytical SDS-PAGE. J Lipid Res.
36. Morrissey JH, Mack GC, Neuenschwander PF, Comp CP. Quantification
of activated factor VII levels in plasma using a tissue factor mutant
selectively deficient in promoting factor VII activation. Blood. 1993;81:
734–744.
Roberts HR. The effect of active site-inhibited factor VIIa on tissue
factor–initiated coagulation using platelets before and after aspirin
38. Zwaal RFA, Comfurius P, Bevers EM. Platelet procoagulant activity and
microvesicle formation: its putative role in hemostasis and thrombosis.
39. Rickles FR, Levin J, Hardin JA, Barr CF, Conrad ME. Tissue factor
generation by human mononuclear cells: effects of endotoxin and disso-
ciation of tissue factor generation from mitogenic response. J Lab Clin
40. Gregory SA, Morrissey JH, Edgington TS. Regulation of tissue factor
gene expression in the monocyte procoagulant response to endotoxin.
41. Mariani G, Bernardi F, Bertina R, Garcia VV, Prydz H, Samama M,
Sandset PM, Di Nucci GD, Testa MG, Bendz B, Chiarotti F, Ciria MV,
Strom R. Serum phospholipids are the main environmental determinants
of activated factor VII in the most common FVII genotype. Haemato-
logica. 1999;84:620–626.
42. Brand K, Banka CL, Mackman N, Tverkjauba RA, Fan S-T, Curtiss LK.
Oxidized LDL enhances lipopolysaccharide-induced tissue factor expres-
sion in human adherent monocytes. Arterioscler Thromb. 1994;14:
790–797.
43. Mészáros K, Aberle S, Dedrick R, Machovic R, Horwitz A, Birr C,
Theofan G, Parent JB. Monocyte tissue factor induction by lipopolysac-
charide (LPS): dependence on LPS-binding protein and CD14, and inhi-
bition by a recombinant fragment of bacterial/permeability-increasing
44. Penn MS, Patel CV, Cui M-Z, DiCorleto PE, Chisolm GM. LDL
increases inactive tissue factor on vascular smooth muscle cell surfaces:
hydrogen peroxide activates latent cell surface tissue factor. Circulation.
Kjalke et al Lipoproteins Enhance Factor VII Activation 1841
Plasma Lipoproteins Enhance Tissue Factor–Independent Factor VII Activation
Marianne Kjalke, Angela Silveira, Anders Hamsten, Ulla Hedner and Mirella Ezban

doi: 10.1161/01.ATV.20.7.1835
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

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/20/7/1835

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/