Activation of Coagulation Factor VII
During Alimentary Lipemia

Angela Silveira, Fredrik Karpe, Margareta Blombäck, George Steiner, Göran Walldius, Anders Hamsten

Abstract
Dietary studies have established a connection between plasma lipoproteins and coagulation factor VII. The present study was undertaken to specifically examine whether factor VII is activated during alimentary lipemia and to investigate the relations of factor VII mass and activity state with fasting and postprandial lipoproteins and free fatty acids (FFAs). Factor VII levels were therefore determined in plasma samples taken before and after intake of a standardized, oral fat load of a mixed-meal type in 33 men (mean age ± SD, 48.8 ± 3.2 years) with a previous myocardial infarction at a young age and 10 healthy, age-matched control subjects. A panel of methods for factor VII determination was used to ensure that changes in all potentially existing forms of the factor during alimentary lipemia would be included. Substantial activation of factor VII was found to occur during alimentary lipemia, whereas the number of factor VII molecules remained constant or even appeared to decrease after the test meal. Activation of factor VII was more pronounced in control subjects than patients, and the proportion of activated factor VII molecules was higher in control subjects. Interestingly, factor VII activation, which correlated quantitatively with the degree of postprandial triglyceridemia, seemed to be related to FFA production during lipolysis of triglyceride-rich lipoproteins that were generated in response to fat intake. Postheparin plasma lipoprotein lipase activity was lower in patients, which could offer one explanation why factor VII activity was lower during alimentary lipemia in these subjects despite their exaggerated postprandial triglyceridemia.

Thus, activation of coagulation factor VII during alimentary lipemia may result in a procoagulant state that is likely to promote the formation of a coronary thrombus in individuals with established coronary artery disease. (Arterioscler Thromb. 1994;14:60–69.)

Key Words • factor VII • alimentary lipemia • free fatty acids • postprandial lipoproteins

Epidemiological, clinical, and experimental studies have suggested that hypertriglyceridemia represents a procoagulant state involving derangements of both blood coagulation and fibrinolysis, particularly because of concomitant elevations of plasma levels of factor VII clotting activity (VIIc) and plasminogen activator inhibitor–1.1 These findings have potential clinical significance, since it could be inferred that the much-debated link between hypertriglyceridemia and coronary heart disease (CHD) might be partly accounted for by disturbances of the hemostatic system. Accordingly, the prospective Northwick Park Heart (NPH) Study has indicated that VIIc is independently associated with the risk of future CHD in middle-aged men and that both serum cholesterol and triglyceride (TG) concentrations are positively correlated with the VIIc level.2,3 Dietary studies have placed additional emphasis on the connection between plasma lipoproteins and factor VII. Addition of fat to the diet has been shown to cause a rapid increase in VIIc.4 The character of the VIIc response to fat intake has suggested that an association with postprandial lipemia exists and that the activity state rather than the plasma concentration of the protein itself is affected. This has been substantiated in subsequent studies of diet-induced hyperlipidemia in the cholesterol-fed rabbit, a condition that largely affects the large lipoprotein particles in the chylomicron and very-low-density lipoprotein (VLDL) fractions and produces high VIIc levels because of activation of factor VII.5 In an epidemiological setting, VIIc has also been shown to be associated with plasma concentrations of large, TG-rich lipoprotein particles.6 Miller et al7 have recently demonstrated that changes in plasma TG concentration throughout the day are positively related to changes in VIIc occurring about 160 minutes later. They have concluded that TG-rich lipoproteins exert a short-term but evanescent effect on factor VII reactivity, irrespective of the lipoprotein lipid-core composition.7 In contrast a long-term increase in VIIc, such as occurs in hypertriglyceridemia, appears to be associated with a rise in factor VII protein concentration.8

The physiology of the factor VII system is intricate, not least because factor VII has the potential to exist in several forms. The zymogen single-chain protein may have some biological activity, but factor VII activity largely resides in the double-chain form.9 Activation of factor VII is generally achieved by formation of a factor VIIa/tissue factor complex that initiates blood coagulation by subsequent activation of factors IX and X.10,11 Activation of blood coagulation by formation of the factor VIIa/tissue factor complex is counteracted by a serine protease inhibitor that is now called tissue factor pathway inhibitor (TFPI).12–14 which is to a major extent associated with low-density lipoprotein (LDL). TFPI provides feedback inhibition of the factor VIIa/tissue...
Table 1. Fasting Levels of Plasma Lipoproteins and Postheparin Lipoprotein Lipase Activity in Normotriglyceridemic and Hypertriglyceridemic Patients and Normolipidemic Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>NTG Patients (n=16)</th>
<th>HTG Patients (n=17)</th>
<th>Control Subjects (n=10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.36±0.16</td>
<td>1.31±0.79†§</td>
<td>0.27±0.18</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL</td>
<td>4.55±0.84</td>
<td>4.18±0.68</td>
<td>4.06±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>1.08±0.35§</td>
<td>0.85±0.14†</td>
<td>1.36±0.38</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.74±0.30</td>
<td>2.64±1.66††</td>
<td>0.56±0.25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL</td>
<td>0.47±0.13</td>
<td>0.49±0.20</td>
<td>0.37±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>0.12±0.05</td>
<td>0.14±0.04</td>
<td>0.11±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>PhPI LPL, mU/mL</td>
<td>431±146</td>
<td>303±80§</td>
<td>483±180</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

NTG indicates normotriglyceridemic; HTG, hypertriglyceridemic; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and PhPI LPL, postheparin plasma lipoprotein lipase activity. Values are mean±SD.

*Analysis of variance. Group differences were identified by the Scheffe F test: †P<.01 compared with control; ‡P<.001 compared with NTG patients; §P<.05 compared with control; ||P<.05 compared with NTG patients.

Factor VII can also be activated by factor Xlla, which is generated on activation of the contact coagulation system. In contrast to double-chain factor Vila, single-chain factor VII shows very limited activity toward its substrates, factors IX and X, in the presence of tissue factor.

"Priming" of the extrinsic coagulation pathway through generation of some factor Vila by the contact coagulation system may serve to promote the expression of clotting activity.

The present study directly addressed the issues of whether factor VII activation takes place during alimentary lipemia in humans and by which mechanism(s). Factor VII antigen and activity levels were determined before and after an oral fat load in patients with premature CHD and control subjects and were related to plasma concentrations of postprandial lipids and lipoproteins and levels of free fatty acids (FFAs) during the test.

Methods

Subjects

A total of 33 patients and 10 control subjects were enrolled in the study. All patients (mean age±SD, 48.8±3.2 years) had survived a first myocardial infarction before the age of 45 years and had subsequently participated in an angiographic study of the mechanisms associated with the progression of coronary atherosclerosis. The study group comprised 13 normolipidemic subjects, 3 normotriglyceridemic subjects with sporadic hypercholesterolemia (normotriglyceridemic [NTG] patients), and 17 subjects with mild to moderate hypertriglyceridemia (hypertriglyceridemic [HTG] patients). The control group comprised men (mean age±SD, 49.2±3.6 years) recruited from participants in a previous population survey. Details of recruitment procedures for patients and control subjects and their clinical characteristics have been published.

Table 2. Free Fatty Acid Responses to Oral Fat Intake in Normotriglyceridemic and Hypertriglyceridemic Patients and Normolipidemic Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>NTG Patients (n=16)</th>
<th>HTG Patients (n=17)</th>
<th>Control Subjects (n=10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA, μmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>679±280</td>
<td>616±201</td>
<td>591±207</td>
<td>NS</td>
</tr>
<tr>
<td>0 Hour</td>
<td>625±204</td>
<td>654±218</td>
<td>497±130</td>
<td>NS</td>
</tr>
<tr>
<td>6 Hour</td>
<td>93±43</td>
<td>93±39</td>
<td>86±16</td>
<td>NS</td>
</tr>
<tr>
<td>18:2</td>
<td>227±66</td>
<td>247±53</td>
<td>175±51</td>
<td>NS</td>
</tr>
<tr>
<td>Δ0−6 Hour</td>
<td>133±56</td>
<td>154±48</td>
<td>89±36</td>
<td>NS</td>
</tr>
<tr>
<td>FFA/PL ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total/PL, 6 hour</td>
<td>203±84</td>
<td>176±45</td>
<td>158±35</td>
<td>NS</td>
</tr>
<tr>
<td>18:2/PL, 6 hour</td>
<td>72±20</td>
<td>67±14</td>
<td>56±13</td>
<td>NS</td>
</tr>
<tr>
<td>Total/PL SI 20-400, 6 hour</td>
<td>1358±637</td>
<td>561±256††</td>
<td>1291±863</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>18:2/PL SI 20-400, 6 hour</td>
<td>471±174</td>
<td>237±122§</td>
<td>342±101</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

NTG indicates normotriglyceridemic; HTG, hypertriglyceridemic; FFA, free fatty acids; 18:2, linoleic acid; and PL, phospholipids. Values are mean±SD.

*Analysis of variance. Group differences were identified by the Scheffé F test: †P<.01 compared with control; ‡P<.001 compared with NTG patients; §P<.05 compared with NTG patients.
At the time of their myocardial infarction all patients were instructed to follow a diet low in fat, rich in complex carbohydrates, and with a limited intake of alcohol. The percentage composition of the different sources of energy in the recommended diet was 10% to 15% protein, 30% fat, and the remainder from carbohydrates. The ratio of saturated to monounsaturated to polyunsaturated fat was 1:1:1.

### Oral Fat-Tolerance Test

All participants were admitted early in the morning to the Clinical Research Unit for a mixed-meal type of oral fat-tolerance test. They had been fasting for 12 hours and been asked to refrain from smoking during the fasting period and from alcohol during the preceding 3 days. Participants were allowed to be ambulatory and to drink water throughout the test. Food and smoking were prohibited.

### Blood Sampling

Blood samples for factor VII and TFPI analyses were obtained by antecubital venipuncture with a 1.4-mm Wasser- man needle (1.4×45 mm; TSK Laboratories, Tokyo, Japan) and for lipoprotein determinations through an indwelling catheter in the other arm. Samples were drawn before intake of the test meal (fasting sample), hourly for the first 9 hours after the test meal, and 12 hours after the test meal. Venous blood was drawn into plastic tubes containing 0.129 mol/L trisodium citrate for the coagulation analyses and into pre-cooled sterile tubes containing 0.34 mol/L tripotassium EDTA (Vacutainer, Becton Dickinson, Meylan, France) for lipid and lipoprotein analyses. Procedures for blood sampling and preparation of citrated plasma samples have been described in detail. The tubes containing blood for lipid and lipoprotein analyses were immediately put into an ice-water bath. Plasma was prepared within 30 minutes by low-speed centrifugation (1750g, 20 minutes, 1°C) and kept at this temperature throughout the preparation procedures. Sodium azide (1.0 mol/L) and the protease inhibitors phenylmethylsulfonyl fluoride (10 mmol/L, dissolved in isopropanol; Sigma Chemical Co, St Louis, Mo) and aprotinin (1400 μg/mL; TrasyloL, Bayer, Leverkusen, FRG) were immediately added to the isolated plasma before fractionation of TG-rich lipoproteins to final concentrations of 1.0 mmol/L, 10 μmol/L, and 28 μg/mL, respectively.

### Factor VII Assays

Coagulation factor VII was assessed in plasma samples drawn before ingestion of the test meal and 3, 6, and 12 hours thereafter. VIIc was determined as described in an LODE coagulometer (Groningen, the Netherlands). Briefly, 100 μL of diluted plasma sample, 100 μL of factor VII-deficient plasma (Helena Laboratories, Beaumont, Tex), and 100 μL of human brain thromboplastin (prepared according to Owren and Aas) were incubated together at 37°C for 30 seconds. Calcium, 100 μL of a 33 mmol/L solution, was added and the clotting time recorded. Factor VIIa was determined with either Thrombotest (a bovine brain thromboplastin preparation that also contains adsorbed bovine plasma; Nyegaard & Co, Oslo, Norway) (VIIa:A) or bovine brain thromboplastin (a gift from Dr Ken Denson, Diagnostic Reagents Ltd, Thames, Oxon, UK) (VIIa:B) in clotting assays that were otherwise essentially as described above. Factor VIIa amidolytic activity (VIIam) was determined with a commercially available kit.

### Table 3. Fasting Factor VII and TFPI Levels in Normotriglyceridemic and Hypertriglyceridemic Patients and Normolipidemic Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>NTG Patients (n=16)</th>
<th>HTG Patients (n=17)</th>
<th>Control Subjects (n=10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII:Ag, U/mL</td>
<td>1.02±0.36</td>
<td>1.29±0.033†</td>
<td>1.22±0.20</td>
<td>.05</td>
</tr>
<tr>
<td>VIIam, U/mL</td>
<td>1.06±0.32</td>
<td>1.36±0.31†</td>
<td>1.22±0.22</td>
<td>.05</td>
</tr>
<tr>
<td>VIIc, U/mL</td>
<td>1.03±0.24</td>
<td>1.16±0.27</td>
<td>1.24±0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Vila:A, U/mL</td>
<td>1.14±0.48</td>
<td>1.12±0.35</td>
<td>1.41±0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Vila:B, U/mL</td>
<td>1.06±0.32</td>
<td>0.97±0.42†</td>
<td>1.42±0.49</td>
<td>.05</td>
</tr>
<tr>
<td>Vila:B/VIIc</td>
<td>1.03±0.23</td>
<td>0.89±0.28†</td>
<td>1.13±0.23</td>
<td>.05</td>
</tr>
<tr>
<td>Vila:B/VII:Ag</td>
<td>1.08±0.31</td>
<td>0.78±0.35†</td>
<td>1.16±0.33</td>
<td>.01</td>
</tr>
<tr>
<td>TFPI, U/mL</td>
<td>1.10±0.41</td>
<td>1.31±0.41†</td>
<td>0.73±0.35</td>
<td>.01</td>
</tr>
</tbody>
</table>

TFPI indicates tissue factor pathway inhibitor; NTG, normotriglyceridemic; HTG, hypertriglyceridemic; VII:Ag, factor VII antigen; VIIam, factor VII amidolytic activity; VIIc, factor VII clotting activity; Vila:A, activated factor VII as determined in an assay with a bovine brain thromboplastin preparation that contains adsorbed bovine plasma; and Vila:B, activated factor VII as determined in an assay with bovine brain thromboplastin. Values are mean±SD.

*Analysis of variance. Group differences were identified by the Scheffé F test: †P<.05 compared with NTG patients; ‡P<.05 compared with control; §P<.01 compared with control.
TABLE 4. Correlation Coefficients Between Basal Levels of Factor VII Antigen and Factor VII Activities and Fasting Lipoprotein Concentrations in Patients

<table>
<thead>
<tr>
<th></th>
<th>VII:Ag</th>
<th>VIIam</th>
<th>VIlc</th>
<th>VIIa:A</th>
<th>VIIa:B</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>0.57‡</td>
<td>0.55‡</td>
<td>0.39*</td>
<td>0.05</td>
<td>-0.27</td>
</tr>
<tr>
<td>Tg</td>
<td>0.52‡</td>
<td>0.50‡</td>
<td>0.33</td>
<td>0.02</td>
<td>-0.29</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>0.03</td>
<td>-0.06</td>
<td>-0.02</td>
<td>-0.07</td>
<td>0.18</td>
</tr>
<tr>
<td>Tg</td>
<td>0.28</td>
<td>0.24</td>
<td>0.25</td>
<td>-0.13</td>
<td>-0.12</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol</td>
<td>0.07</td>
<td>0.09</td>
<td>0.07</td>
<td>0.13</td>
<td>0.32</td>
</tr>
<tr>
<td>Tg</td>
<td>0.32</td>
<td>0.24</td>
<td>0.15</td>
<td>-0.14</td>
<td>-0.11</td>
</tr>
<tr>
<td>Sf 60-400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>0.34*</td>
<td>0.35</td>
<td>0.28</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>B-100</td>
<td>0.54†</td>
<td>0.54‡</td>
<td>0.31</td>
<td>0.02</td>
<td>-0.22</td>
</tr>
<tr>
<td>Sf 20-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>0.40*</td>
<td>0.42*</td>
<td>0.34</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>B-100</td>
<td>0.47†</td>
<td>0.55‡</td>
<td>0.36*</td>
<td>0.15</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

VII:Ag indicates factor VII antigen; VIIam, factor VII amidolytic activity; VIlc, factor VII clotting activity; VIIa:A, activated factor VII as determined in an assay with a bovine brain thromboplastin preparation that contains adsorbed bovine plasma; VIIa:B, activated factor VII as determined in an assay with bovine brain thromboplastin; VLDL, very-low-density lipoprotein; Chol, cholesterol; Tg, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Sf, Svedberg flotation rate; B-48, apolipoprotein B-48; and B-100, apolipoprotein B-100.

*P<.05, †P<.01, ‡P<.001.

Lipoprotein Fractionation

TG-rich lipoproteins (chylomicrons, VLDL, and their respective remnants) were fractionated from plasma samples drawn before ingestion of the test meal and 3, 6, and 12 hours thereafter by cumulative rate ultracentrifugation. Consecutive runs calculated to float Sf >400, Sf 60 to 400, and Sf 20 to 60 particles were performed. After each centrifugation the top 0.5 mL of the gradient containing the respective lipoprotein subclasses was aspirated and the tube refilled with <f=1.006 kg/L salt solution before the next run. The Sf 12 to 20 fraction was recovered after the last ultracentrifugation run by slicing the tube 29 mm from the top after the Sf 20 to 60 lipoproteins had been aspirated. Aliquots of the three subfractions of TG-rich lipoproteins were immediately placed under nitrogen and frozen at -80°C for later determination of apolipoprotein (apo) B-48 and B-100. The Sf 12 to 20 fraction was recovered after the last ultracentrifugation run by slicing the tube 29 mm from the top after the Sf 20 to 60 lipoproteins had been aspirated. Aliquots of the three subfractions of TG-rich lipoproteins were immediately placed under nitrogen and frozen at -80°C for later determination of apolipoprotein (apo) B-48 and B-100. The Sf 12 to 20 subfraction was dialyzed extensively against 0.15 mol/L NaCl, 0.27 mmol/L Na₂EDTA, and 1.5 mmol/L NaN₃ at 2°C and then frozen.

The major plasma lipoprotein classes (TG-rich lipoproteins [d<1.006 kg/L], LDL, and high-density lipoproteins [HDLs])

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**FIG 2.** Scattergrams showing relations of very-low-density lipoprotein (VLDL) triglyceride (Tg) to factor VII antigen (ag) (left) and factor VII amidolytic activity (am) (right) in control and patient groups. Correlation coefficients between VLDL Tg and Vilag were 0.43 (NS) for the control group (n=10; o) and 0.52 (P<.01) for the patient group (n=33; e). Corresponding correlation coefficients for VIIam were 0.75 (P<.01) for the control group and 0.50 (P<.01) for the patient group.
FIG 3. Scattergram showing the relation of high-density lipoprotein cholesterol (HDL Chol) to factor VIIa:A in control and patient groups. Correlation coefficients between HDL Chol and VIIa:A were .81 (P<.01) for the control group (n=10, o) and .13 (NS) for the patient group (n=33, •).

were also determined on samples obtained before the test meal and 3, 6, and 12 hours after the meal by a combination of preparative ultracentrifugation and precipitation of apo B-containing lipoproteins followed by lipid analysis.26

**Determination of Apo B-48 and B-100**

Apo B-48 and B-100 were quantified with an assay that is based on the relative chromogenicities of these apolipoproteins after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).27 Aliquots of freshly isolated and processed lipoprotein samples containing 200 to 500 μg of total protein28 were delipidated.29 The delipidated precipitate was dissolved overnight in sample buffer (50 mmol/L Na2PO4, 1% SDS, and 1% β-mercaptoethanol, pH 7.2) and applied to 3.3% SDS-PAGE rod gels. Details for the procedure as well as calculation of relative masses of apo B-100 and B-48 have been published.19 The absolute masses of apo B-100 and B-48 were derived from their relative quantities and the total apo B mass. The latter was quantified in all fractions of Sf>12 lipoproteins by electroimmunoassay after pretreatment of samples with lipase.30

**Lipid Analysis**

Total cholesterol and TGs were determined in triplicate in plasma and in the major plasma lipoproteins after extraction with chloroform/methanol.31 Cholesterol32 and TGs33 were then determined on an Ultraspec (Pharmacia LKB Biotecnology AB, Uppsala, Sweden).

TGs, cholesterol, and phospholipids in isolated fractions of TG-rich lipoproteins were determined by enzymatic methods (877577; Boehringer Mannheim Diagnostica, Mannheim, FRG; 14106-14108, Merck Diagnostica, Darmstadt, FRG; and 990-54008 Wako Chemicals, Neuss, FRG, respectively).

**Lipoprotein Lipase (LPL) Activity**

An intravenous injection of heparin (100 U/kg body weight) was given on a separate visit 1 week after the oral fat-tolerance test. Determination of plasma LPL activity in fasting venous blood before and after heparin injection was performed as published34 under the same assay conditions as described by Bengtsson-Olivecrona and Olivecrona.34 LPL activity was expressed in milliunits per milliliter, which corresponds to 1 nmol of fatty acid released per minute per volume of postheparin plasma.

**Analysis of FFAs**

Total plasma FFAs were determined according to Ho35 after extraction according to Dole.36 The fatty acid composition of the FFAs was determined by gas-liquid chromatography after preparative thin-layer chromatography of FFAs from a total lipid extract. Details of this method have been described.20 Because of technical problems linoleic (18:2) FFA was measured in only 24 patients and 5 control subjects.

**Statistical Analysis**

Conventional methods were used for calculation of means, SDs, and SEMs. Coefficients of skewness and kurtosis were calculated to test deviations from a normal distribution. Logarithmic transformation was performed on individual values of the skewed variables, and a normal distribution of values was confirmed before statistical computations and significance
testing. Statistical significance for differences in continuous variables between more than two groups was tested by one-way ANOVA. The Scheffé F test was employed to identify differences among groups when the overall F statistic was significant. Within-group comparisons of measurements made at various times during the oral fat-tolerance test were done by Student's paired t test. Pearson correlation coefficients were calculated between factor VII variables and plasma lipids, lipoproteins, and FFAs. Multiple stepwise linear regression analysis was performed to determine the independent relations between changes in plasma lipids, lipoproteins, or FFAs and factor VII activation during alimentary lipemia. The variable with the highest partial correlation coefficient was entered at each step until no variable remained with an F value (F to enter) of 4 or more.

Because of the small size of the study groups and the multitude of comparisons, only group differences or correlations with P<.01 were considered as major findings and therefore taken as a basis for discussion.

Ethical Considerations

The experimental protocol was approved by the ethics committee of the Karolinska Hospital, Stockholm, Sweden. All subjects gave their informed consent to participate in the study.

Results

Fasting and Postprandial Lipids and Lipoproteins

Fasting plasma lipoprotein levels; responses of lipids, lipoproteins, and FFAs to the oral fat load; and post-heparin plasma LPL activity in patients and control subjects are shown in Tables 1 and 2 and Fig 1 and have been described in detail.18,19 Plasma TGs increased significantly in all groups in response to the test meal (Fig 1). Peak levels were attained after 3 hours in the control subjects, whereas these levels were delayed until 5 hours in both patient groups. For phospholipids the peak plasma level appeared to be delayed in HTG patients only. Otherwise a pattern similar to that of plasma TGs was seen.

The total FFA content in plasma did not increase in response to the test meal, whereas the 18:2 FFA concentration increased in all groups (Table 2). The rise in 18:2 FFA tended to be higher in patients. Ratios between FFAs and phospholipids in whole plasma and SF 20 to 400 lipoproteins were calculated as a measure of the density of FFAs on the surfaces of TG-rich lipoproteins. The ratios of total and 18:2 FFAs to SF 20 to 400 phospholipids were considerably lower in HTG patients.

Basal Factor VII and TFPI Levels in Patients and Control Subjects

Basal (fasting) VIIc levels were similar in HTG patients and control subjects but tended to be lower than control levels (NS) in the NTG patient group (Table 3). VIIam and VIIc activities also showed the same pattern. Factor VIIa levels (measured with the two bovine thromboplastin preparations) tended to be lower in both patient groups than control subjects. The VIIa:B to VIIc and VIIa:B to VII:Ag ratios were accordingly lower in both patient groups than in control subjects. Differences between the NTG and HTG patient groups were significant for VII:Ag and VIIam, with lower values in the NTG group, and for the VIIa:B to VIIc and VIIa:B to VII:Ag ratios, which in contrast were lower in the HTG patient group.

Basal TFPI levels were increased in both patient groups, the difference between HTG patients and control subjects being statistically significant (Table 3).

Correlations Between Basal Factor VII Variables and Fasting Plasma Lipoproteins

Table 4 shows correlation coefficients between levels of factor VII variables and plasma lipoproteins measured in fasting samples in the patient group. Fairly strong positive correlations were found between fasting levels of VII:Ag and VIIam and the VLDL cholesterol and TG concentrations, whereas corresponding associations for factor VII activity measurements were weaker and statistically significant only for VIIc. No apparent associations were noted between factor VII measurements and LDL or HDL lipid levels.

Computation of correlation coefficients with subfractions of TG-rich lipoproteins measured in fasting plasma indicated that the concentrations of both chylomicron remnants (apo B-48) and VLDL (apo B-100) in

![Figure 5](http://atvb.ahajournals.org/)

Figure 5. Line plot showing responses of factor VIIa:B and 18:2 (linoleic acid) free acid (FFA) to the oral fat load in patients. Values are given as mean±SEM. Levels of factor VIIa:B (●) and 18:2 FFA (□) increased in parallel, with peak values attained in the 6-hour sample. a:B indicates activated factor VII as measured with bovine brain thromboplastin.

Table 5. Correlation Coefficients Between the 0- to 6-Hour Increase in Plasma Lipids, Lipoproteins, and 18:2 Free Fatty Acid in Response to Oral Fat Intake and the Corresponding Increase in Factor VII Activity in Patients

<table>
<thead>
<tr>
<th></th>
<th>∆VIIc</th>
<th>∆VIIa:B</th>
<th>∆VIIa:A</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆Tg</td>
<td>0.35*</td>
<td>0.52†</td>
<td>0.43*</td>
</tr>
<tr>
<td>∆PL</td>
<td>0.42*</td>
<td>0.47†</td>
<td>0.54†</td>
</tr>
<tr>
<td>∆SF 60-400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>0.24</td>
<td>0.36*</td>
<td>0.39*</td>
</tr>
<tr>
<td>B-100</td>
<td>0.39*</td>
<td>0.47†</td>
<td>0.32</td>
</tr>
<tr>
<td>∆SF 20-60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>0.46†</td>
<td>0.12</td>
<td>-0.01</td>
</tr>
<tr>
<td>B-100</td>
<td>0.01</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>∆18:2 FFA</td>
<td>0.46*</td>
<td>0.38</td>
<td>0.24</td>
</tr>
</tbody>
</table>

VIIc indicates factor VII clotting activity; VIIa:A, activated factor VII as determined in an assay with a bovine brain thromboplastin preparation that contains adsorbed bovine plasma; VIIa:B, activated factor VII as determined in an assay with bovine brain thromboplastin; Tg, triglycerides; PL, phospholipids; SF, Svedberg flotation rate; B-48, apolipoprotein B-48; B-100, apolipoprotein B-100; and FFA, free fatty acid.

*P<.05, †P<.01.
the Sf 20 to 400 range correlated with VII:Ag and VIIam levels. Correlations of borderline statistical significance were also observed with VIIc. Associations with VII:Ag and VIIam tended to be stronger for VLDL than for chylomicron remnants.

The relations of VLDL TGs with VII:Ag and VIIam were apparent in both patients and control subjects, with a steeper slope for the regression line in the control group (Fig 2). Correlation coefficients for VLDL TGs versus VII:Ag were 0.43 (NS) for control subjects and 0.52 (P < 0.01) for both patient groups. Corresponding values for VIIam were 0.75 (P < 0.01) for control subjects and 0.50 (P < 0.01) for patients. Associations similar to those observed in patients were also suggested for the various subfractions of TG-rich lipoproteins, particularly with VIIam, but because of the limited number of control subjects statistical significance was not always achieved. A striking positive relation was observed between fasting HDL cholesterol concentration and the VIIa:A level in control subjects (r = 0.81, P < 0.01), which was not apparent among patients (r = 0.13, NS), whose range of HDL cholesterol values was narrow (Fig 3).

Factor VII Variables and TFPI During Alimentary Lipemia

Factor VII antigen and activities in blood samples taken before and 3, 6, and 12 hours after the test meal are illustrated in Fig 4. Compared with fasting levels, VII:Ag and VIIam tended to be lower in the 3-hour sample in both patients and control subjects and gradually returned to baseline toward the end of the 12-hour period. Interestingly, this decrease in both VII:Ag and VIIam in the 3-hour sample (P < 0.001) appeared to be more consistent among HTG patients. In contrast, levels of VIIc as well as factor VIIa increased in all groups, with peak values attained in the 6-hour sample and a return to baseline levels in the 12-hour sample. The most pronounced increase compared with baseline values was observed for VIIa:B in control subjects, in whom substantial VIIa:B elevations were already seen in the 3-hour sample. Plasma TFPI activity levels did not change in any subject group during alimentary lipemia (data not shown).

Relations of TG-Rich Lipoproteins to Factor VII Variables During Alimentary Lipemia

Relations between TG-rich lipoproteins, 18:2 FFAs, and factor VII activity during alimentary lipemia in patients are shown in Fig 5 and Table 5. VIIa:B appeared to increase in parallel with 18:2 FFA (Fig 5). As shown in Figs 1 and 4, VIIc, VIIa:A, and VIIa:B increased substantially, particularly in control subjects, with peak values attained after the TG peak, which was reached at 3 hours in control subjects but delayed until 5 hours in both patient groups.

Table 5 shows correlation coefficients between the 0- to 6-hour increase in plasma TGs, phospholipids, B apolipoproteins, and 18:2 FFAs in response to the oral fat load and the corresponding increase in factor VII activity in patients. The rise in plasma TG and phospholipid levels was consistently correlated with the increase in factor VII activity. Increases in the large lipoprotein particle species, the Sf 60 to 400 chylomicron remnants, and Sf 60 to 400 VLDL also appeared to be related to factor VII activation. Except for a significant correlation between the smaller Sf 20 to 60 chylomicron remnants and VIIc, no associations were found between the responses of the smaller, TG-rich lipoprotein particles and factor VII activity. Of note, an increase in 18:2 FFA correlated significantly with the VIIc response.

To further determine the relations between alimentary lipemia and the activation of coagulation factor VII, stepwise multiple regression analyses were performed for the patient group, with the 0- to 6-hour changes in VIIa:B and VIIc as dependent variables. In the first step basal TFPI activity was entered as a forced variable to control for between-individual differences in TFPI, increases between baseline and 6-hour values in plasma TGs and phospholipids, large and small chylomicron remnants, and VLDL; 18:2 FFAs; and basal VII:Ag were used as independent variables. In this model an increase in plasma TGs alone was found to be significantly related, independently of TFPI, VII:Ag, other lipoprotein variables, and 18:2 FFA, to VIIa:B (increase in multiple R² = 0.25), and to VIIc (increase in multiple R² = 0.34).

Discussion

In the past few years interest has increased considerably in the possible interactions between plasma lipoproteins and several components of the hemostatic system. There is now consistent epidemiological, clinical, and experimental evidence that hypertriglyceridemia may represent a procoagulant state involving derangements of both blood coagulation and fibrinolysis. The present study specifically addressed the issue of the activation of factor VII during alimentary lipemia in humans and examined the relations of factor VII mass and activity state of the molecule with fasting and postprandial lipoproteins and FFAs. To ensure that changes in all potentially existing forms of factor VII during alimentary lipemia would be accounted for in this study, a panel of methods was used for factor VII determination.37 Substantial activation of factor VII was found to occur during alimentary lipemia, whereas the number of factor VII molecules, as reflected by VII:Ag measurements, remained constant or even appeared to decrease after the test meal. Activation was preceded by postprandial triglyceridemia, which correlated quantitatively with the degree of factor VII activation. Interestingly, activation of factor VII seemed to be related to FFA production during lipolysis of TG-rich lipoproteins that were generated in response to fat intake. The plasma levels of fasting TG-rich lipoproteins were also associated with basal factor VII protein levels. TFPI activity levels were higher in HTG patients, but no changes were seen during alimentary lipemia in any group of subjects. This means that tissue factor–induced activation of factor VII may be more efficiently countered in HTG patients.

It is noteworthy that activation of factor VII was more pronounced in control subjects than patients and that the proportion of activated factor VII molecules was higher in control subjects. Thus, in contrast to earlier studies,38,29 basal or postprandial hypertriglyceridemia was not associated with an elevated basal factor VII activity. However, there are previous reports of increased factor VII mass, lower factor VII activity, and a decreased proportion of factor VIIa in patients with
coronary artery disease. A recent study of familial LPL deficiency has clearly demonstrated that high plasma concentrations of TG-rich lipoproteins will increase VIIc only in the presence of LPL. Postheparin plasma LPL activity, which is assumed to reflect the LPL available at the endothelial surface, was lower in HTG patients, which could explain why factor VII activity was lower in these subjects despite elevated fasting and postprandial levels of TG-rich lipoproteins.

The observation that micellar, long-chain fatty acids can stimulate activation of factors XII and VII in vitro when added to human plasma further attests to the hypothesis that generation of FFAs from chylomicrons and VLDL during lipolysis is involved in the activation of the contact coagulation pathway. The strong, positive association between plasma HDL cholesterol concentration and factor VII activity in control subjects might also suggest that lipolysis of TG-rich lipoproteins and generation of FFAs are important events in regulating factor VII activity. A positive albeit weaker correlation between factor VII activity and HDL cholesterol concentration has been observed in healthy young adults. Differences in dietary habits between groups are unlikely to account for basal differences in the state of activation of factor VII, since changes in the amount and quality of fat have been shown not to affect the proportion of factor VIIa molecules. It should be noted in this context that activation might vary depending on the chain length and degree of saturation of the fatty acids in the test meal. However, activation of factor VII by postprandial triglyceridemia has also been reported to be uninfluenced by dietary fat composition.

The molecular mechanisms underlying possible activation of factor VII by TG-rich lipoproteins remain to be elucidated. However, a possible explanation, based on in vitro experiments and studies with the hypercholesterolemic rabbit, might be that large lipoprotein particles such as chylomicrons, VLDL, and their remnants that carry the appropriate FFAs at a sufficient density of negative charge could activate the intrinsic coagulation pathway and thereby factor VII via factor XIIa, with conversion of zymogen single-chain factor VII to two-chain VIIa. The generation and subsequent transfer of FFAs from the TG core to the phospholipid surface of large, TG-rich lipoproteins through the action of LPL are likely to have a central role in activation of the contact coagulation pathway. This sequence of events would accord with the findings in the present study. First, postprandial triglyceridemia preceded the activation of coagulation factor VII, and the increase in plasma TGs and the larger species of TG-rich lipoproteins correlated quantitatively with the degree of factor VII activation. Second, efficient lipolysis of TG-rich lipoproteins promotes factor VII activation, which may also partially explain why the activity state differed between patients and control subjects. Increases in linoleic (18:2) FFA, which reflected lipolysis of newly synthesized chylomicrons and chylomicron remnants because of the composition of the test meal, were associated with factor VII activation. Third, the greater increase in TG-rich lipoproteins in response to the fat load in HTG patients, who had lower LPL activity, resulted in a lower than normal density of FFAs on the lipoprotein surface as indicated by the reduced ratio of total and 18:2 FFAs to plasma phospholipids. These lipoproteins consequently constituted a poorer contact surface and generated less factor XIIa and IXa, the products of contact activation. Accordingly, despite pronounced postprandial triglyceridemia, HTG patients had lower factor VII activity and a smaller proportion of factor VIIa molecules than healthy, normolipidemic subjects.

An alternative proposal is that the positive association between plasma TGs and factor VII activity is due to binding of the coagulation protein to TG-rich lipoprotein particles, with an ensuing decrease in its fractional catabolic rate, particularly in subjects with prolonged alimentary lipemia. Indeed, in vitro experiments have shown that factor VII binds to all apo B-containing lipoproteins but not to HDL and that the binding is stronger to chylomicron remnants or VLDL. Tissue factor associated with lipoproteins has been suggested to be involved in this interaction, which may generate factor VII activity. However, binding of factor VII molecules to TG-rich lipoproteins has not been demonstrated in vivo. Furthermore, factor VII protein concentrations were not increased in the present study, which would argue against decreased catabolism as an explanation of factor VII activation during alimentary lipemia.

It has been suggested that some TG-rich lipoproteins bind zymogen single-chain factor VII and enhance its catalytic activity without conversion to two-chain factor VIIa. This hypothesis was advanced to explain the discrepant results obtained in coronary patients regarding the comparison between VIIc and factor VII activity as determined by an assay sensitive to factor VIIa. Because zymogen single-chain factor VII has measurable catalytic activity, it was considered reasonable to assume that a complex of lipoprotein and zymogen factor VII could possess enhanced activity (detected as VIIc) by virtue of a conformational change in factor VII. This line of reasoning would also agree with the significant positive correlations obtained between plasma levels of TG-rich lipoproteins and VIIc values in the present study. This hypothesis is also thought to explain the increased plasma levels of VIIc that have been linked to a fraction of the factor VII molecules that are present in an activated state in phospholipase C-sensitive complexes, hence termed phospholipid-factor VII complexes. Like VIIc, plasma levels of the phospholipid-factor VII complex correlate directly with plasma TG concentration. Recently a direct relation has been suggested between the level of phospholipid-factor VII complex in plasma and the dietary influence on plasma TG concentration.

The associations between fasting and postprandial plasma levels of TG-rich lipoproteins and factor VII protein in the present study are likely to be secondary to long-term activation of factor VII. Factor VII activity is indicated to be directly correlated with the rate of conversion of the procoagulant zymogens factor IX, factor X, and prothrombin to their active enzymes, with the resulting release of their respective activation peptides. The prothrombin activation peptide has been shown to regulate the synthesis of vitamin K–dependent proteins in rabbits. Thus, TG-rich lipoproteins induce activation of factor VII through contact activation and the intrinsic coagulation pathway, with subsequent release of prothrombin fragments that may exert a positive feedback control of factor VII synthesis in the liver.
Differences in the associations of TG-rich lipoproteins and CHD with factor VII levels between studies may to a large extent depend on the assay techniques employed and the nature of the thromboplastin preparations used. Some of the observed discrepancies are thus likely to derive from the fact that different factor VII–related activities have been measured. Future studies will require more sensitive and specific assays of the factor VIIa molecules in particular, a prerequisite of which is a better basic knowledge of the factor VII/VIIa interaction. Assay standardization procedures are also needed.

A limitation of the present study is the retrospective design, which confers an inherent selection bias, and the small study groups. The possibility exists that patients who did not survive to participate in the present study had different factor VII activity and protein levels. We also selected patients in whom genetic type II hyperlipoproteinemias were not present. Therefore we cannot say whether our results extend to subjects with hypercholesterolemia. Furthermore, our data may not be limited to male patients of this age group with premature coronary artery disease and a previous myocardial infarction. Larger-scale, case-control studies of representative cohorts of patients of both sexes with CHD are warranted in which both factor VII mass and activity state of the molecule are determined.

With these restrictions in mind, the clinical corollary of the present findings is that alimentary lipemia is a procoagulant state that is likely to promote formation of occlusive thrombi on fissured atherosclerotic plaques that evolve through other mechanisms. A hypercoagulable state may also predispose to increased fibrin deposition on injured intimal surfaces, fibrin incorporation, and subsequent evolution and growth of atherosclerotic plaques. Since most of our lives are spent in the postprandial state, disturbances of lipoprotein metabolism leading to postprandial triglyceridemia may thus have both atherogenic and thrombotic consequences. However, before a final assessment can be made of the role of factor VII activation during alimentary lipemia, counteracting and balancing mechanisms within the hemostatic system need to be studied. From a practical point of view, the factor VII activity state of the individual should preferably be evaluated under non-fasting conditions, as was actually done in the NPH study, the only prospective epidemiological study that has shown that factor VII activity is a marker of increased risk for CHD.

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