Activation of the Contact System of Coagulation Does Not Contribute to the Hemostatic Imbalance in Hypertriglyceridemia

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Abstract—In vitro, triglyceride-rich lipoproteins may act as a surface to initiate the contact system of coagulation. Therefore, we studied the activation of factor XII (FXII), prekallikrein, and FXI and the generation of thrombin in 52 hypertriglyceridemic patients before and after 12 weeks of triglyceride-lowering treatment with gemfibrozil or n-3 polyunsaturated fatty acids. Thrombin generation was assessed by measuring the levels of prothrombin fragment F1 + 2 and thrombin-antithrombin (TAT) complexes. Contact activation was assessed by measuring FXIIa, kallikrein, and FXIa in complex with their major inhibitor, C1 inhibitor, and FXIa was also determined as part of a complex with $\alpha_1$-antitrypsin. Triglyceride and cholesterol levels decreased equally in both treatment groups. In the gemfibrozil group, there was a significant decrease in F1 + 2, while TAT complexes did not change. FXIIa- and kallikrein-C1 inhibitor complexes were elevated in 13% and 9% of the patients before treatment, respectively, and no changes were observed on triglyceride-lowering therapy. Also, no significant changes in regard to FXIa–C1 inhibitor and FXIa–$\alpha_1$-antitrypsin complexes were seen. FXIa–$\alpha_1$-antitrypsin complexes were present in 70% of the patients before treatment and were positively correlated with the level of TAT complexes. In conclusion, we did not detect an effect on activation markers of the contact coagulation system in hypertriglyceridemic patients after triglyceride-lowering therapy. Therefore, contact activation is not likely to contribute to the hypercoagulability seen in these patients. (Arterioscler Thromb Vasc Biol. 1999;19:2548-2553.)

Key Words: hypertriglyceridemia $\bullet$ contact system $\bullet$ blood coagulation

The contact system of coagulation consists of the serine proteases factor XII (FXII), factor XI (FXI), prekallikrein (PK), and the cofactor high-molecular-weight kininogen. The role of this system in the initiation of coagulation has been questioned because deficiencies in FXII or PK are not associated with an increased bleeding tendency.1 In addition, the physiological activator or surface for the (auto)activation of FXII is unknown.2 Long-chain, saturated fatty acids have been suggested to provide a potent surface for contact activation.3–5 These fatty acids are present in most lipoproteins and are elevated in patients with hypertriglyceridemia. Little doubt exists that triglycerides are related to atherosclerotic disease. Triglycerides themselves do not accumulate in atherosclerotic lesions, so their atherogenicity must be related to the associated adverse quantitative and qualitative changes in circulating lipoproteins.6

It was demonstrated in several cross-sectional studies that levels of triglycerides are associated with levels of factor VII (FVII) as measured in a clotting assay (FVII-c).7,8 Furthermore, the Northwick Park Heart Study demonstrated that high levels of FVII-c constitute an independent risk factor for acute coronary events.9 Others have suggested that these high levels of FVII-c represent enhanced levels of activated FVII (FVIIa).10 Because FVIIa in complex with tissue factor activates prothrombin, this could explain the hypercoagulability seen in patients with hypertriglyceridemia and thus, the associated higher risk of coronary artery disease.11,12

Because activated FXII (FXIIa) can directly activate FVII in vitro, this activation route has been postulated to explain elevated FVII-c levels in patients with hypertriglyceridemia.5,13–15 FXIIa also activates FXI and thereby, the intrinsic coagulation pathway, leading to the downstream formation of thrombin and fibrin.16 To address the hypothesis that hypertriglyceridemia causes activation of the contact system, we analyzed markers of contact activation in a cohort of males with severe hypertriglyceridemia. Furthermore, we studied the effect on these markers of 2 different triglyceride-lowering interventions in our patients.

Methods

Patients
Male patients from outpatient lipid clinics from the Academic Medical Center and the Slotervaart Hospital in Amsterdam, Nether-
lands, aged 18 to 70 years with severe hypertriglyceridemia, were asked to participate in the study. This study was part of a double-blind treatment study evaluating the effect on serum triglycerides of n-3 polyunsaturated fatty acids (n-3 PUFAs) compared with gemfibrozil. Patients using Coumadin were excluded from analysis. After written, informed consent was obtained, patients followed a diet during a run-in period of 6 weeks, according to the National Guidelines for Healthy Nutrition in the Netherlands. Patients who had a serum triglyceride level of 4.5 mmol/L or higher after this period were randomized to receive either oral therapy with n-3 PUFAs (Omacor), 4 g daily, or gemfibrozil (Lopid), 1200 mg daily, for 12 weeks. All other medications used by the patients were recorded and continued during the trial.

Clinical cardiovascular disease was considered to be present if subjects met at least 1 of the following criteria: (1) if a diagnosis of clinically documented angina pectoris had been made; (2) if subjects had developed a myocardial infarction or if an intervention by either coronary bypass surgery or balloon angioplasty had been performed; (3) if the patients had suffered an ischemic stroke; or (4) if a history of intermittent claudication was present.

Blood Sampling and Assays
After 12 hours, fasting blood samples were taken by venipuncture into siliconized tubes (with 0.34 mmol/L EDTA; Vacutainer, Becton Dickinson) to assess cholesterol and triglyceride levels and into siliconized, citrated tubes (with 0.105 mmol/L sodium citrate; Vacutainer, Becton Dickinson) for detection of thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2 (F1+2). Blood samples for the measurement of FXI, FXII, and PK activation were collected in siliconized tubes to which a solution of 0.1 mmol/L EDTA, 0.1 mg/mL soybean trypsin inhibitor (type I-S from Sigma Chemical Co), and 20 mmol/L benzamidine (Acros; all final concentrations) was added to prevent any ex vivo activation of the clotting system. Platelet-poorn plasma was obtained by centrifugation at 2000g for 20 minutes at room temperature. Plasma samples were stored at −70°C until assayed.

Total cholesterol in plasma was determined by an enzymatic colorimetric procedure with cholesterol esterase.17 Triglycerides were quantified by an enzymatic colorimetric procedure with lipase, glycerokinase, and glycerol-3-phosphate.18 Prothrombin F1 10%.

Blood sampling and assays

| TABLE 1. Patient Characteristics and Laboratory Parameters Before Treatment |
|-----------------------------------------------|---------|---------|
| n-3 PUFAs | Gemfibrozil |     |
| Age, y | n=32 | n=29 | P |
| 50.2±7.7 | 49.8±8.9 | 0.4 |
| Medication, % | 53.1 | 67 | 0.2 |
| Atherosclerosis, % | 21.9 | 13.8 | 0.4 |
| Triglycerides, mmol/L | 13.1±8.6 | 10.6±5.8 | 0.01 |
| Cholesterol, mmol/L | 8.7±2.5 | 7.8±1.7 | 0.06 |
| F1+2, nmol/L | 1.12 (0.68–1.82) | 1.03 (0.37–1.7) | 0.2 |
| TAT, µg/L | 3.8 (1.2–12.5) | 3.3 (0.8–9.6) | 0.5 |
| FXI antigen, nmol/L | 560±170 | 540±170 | 0.6 |
| FXIa–Cl inhibitor, pmol/L | <80 (<80–806) | <80 (<80–211) | 0.1 |
| PK – antilipase, nmol/L | 620±200 | 540±120 | 0.1 |
| Kallikrein–Cl inhibitor, pmol/L | <80 (<80–830) | 102 (<80–733) | 0.9 |
| FXI, nmol/L | 36±9.8 | 32.9±5.7 | 0.05 |
| FXIIa–Cl inhibitor, pmol/L | <10 (<10–57.7) | <10 (<10–301) | 0.6 |
| FXII–α-antitrypsin, pmol/L | 12 (<10–45) | 13 (<10–27) | 0.7 |

Age, triglycerides, cholesterol, FXII, PK, and FXI antigen are presented as mean±SD; coagulation activation markers are presented as median and (range).

complexes were not detectable. FXI antigen was measured with MAb XI-5 on the solid phase and biotinylated MAb XI-3 for detection.24

Statistical Analysis
Results are presented as mean±SD or as median (range). Differences between the 2 treatment groups at the start of the treatment were analyzed by χ^2 or with an unpaired t test. Differences between 0 and 12 weeks were analyzed with a paired-sample t test. Parameters whose values were not normally distributed were analyzed with the Mann-Whitney U test and differences between 0 and 12 weeks with the Wilcoxon rank test. Associations were analyzed by Pearson or Spearman rank correlation analysis and are presented as a correlation coefficient (r). A 2-sided P value of 0.05 was considered statistically significant.

Results
In total, 61 male patients entered the study after the diet phase. Thirty-two patients were randomized to treatment with n-3 PUFAs and 29 patients received gemfibrozil. Patient characteristics and values of cholesterol, triglycerides, and coagulation parameters before treatment are shown in Table 1. Triglyceride levels were significantly different between the study groups (P=0.01).

After 12 weeks of therapy and continuation of the prescribed diet, triglycerides and cholesterol levels had decreased significantly in both groups (Figure 1). In the n-3 PUFA treatment group, triglycerides decreased to 7.6±4.8 mmol/L and in the gemfibrozil group, to 5.6±7.1 mmol/L (P<0.0001 for either group compared with baseline levels). The cholesterol levels decreased to 7.5±1.7 and 6.7±1.9 mmol/L, respectively, in the n-3 PUFA group and the gemfibrozil group (P<0.01 for either group compared with baseline). Although there was a difference in triglyceride and cholesterol levels at the start of treatment, the decreases in triglycerides and cholesterol were comparable in both treatment groups. Triglyceride levels decreased by 5.5±5.4 and 5±6.5 mmol/L in the n-3 PUFA and the gemfibrozil group, respectively, and the cholesterol levels by
patients before therapy and remained elevated in 1. In 4 other patients, kallikrein–C1 inhibitor levels increased to >350 pmol/L after therapy. The FXII antigen and PK antigen levels did not significantly differ after n-3 PUFA therapy. In the gemfibrozil group, a significant increase from baselines values was observed for both FXII and PK antigen levels, to 600±220 nmol/L (P=0.02) and 630±210 nmol/L (P=0.01), respectively (Figure 2).

FXIIa–C1 inhibitor complexes were detectable in only 3 patients before treatment and remained elevated after 12 weeks of therapy in 1 patient receiving gemfibrozil and in another having n-3 PUFA treatment. FXIα–α1-antitrypsin complexes were elevated in 71% of the patients before treatment and remained elevated after treatment in 60% (Table 2, P=NS). FXI antigen increased after therapy, from 34.4±8 to 38.5±9.2 nmol/mL, which was highly significant (P<0.0001). However, the increase in FXI antigen levels only occurred in the group receiving gemfibrozil, from 32.9±5.7 to 41±8 nmol/mL (P<0.0001), while antigen levels did not change after n-3 PUFA therapy (Figure 2).

A small but significant decrease occurred in F1+2 (P=0.02), but TAT complexes were not affected by the triglyceride-lowering therapy (Table 3). The decrease in F1+2 levels occurred mainly in the gemfibrozil group (1.03 nmol/L; range from 0.37 to 1.7 to 0.87 nmol/L before; 0.87 nmol/L; range from 0.17 to 1.23 nmol/L after, P=0.003), whereas F1+2 levels did not change significantly in the n-3 PUFA group.

At the beginning of the study, associations were found between triglyceride and cholesterol levels (r=0.64, P<0.0001), between TAT complexes and F1+2 (r=0.33, P=0.02), and between FXIIa–C1 inhibitor and kallikrein–C1 inhibitor complexes (r=0.96, P<0.001). Triglyceride or cholesterol levels were not correlated with F1+2, TAT complexes, or markers of contact activation. Significant correlations between TAT complexes and FXIIa–α1-antitrypsin complexes were found at the beginning of the study and after therapy (r=0.47, P=0.01, and r=0.4, P=0.04, respectively).

Eleven patients (18%) had a history of clinical cardiovascular disease. These patients had similar triglyceride and cholesterol levels. Markers of activation of coagulation in these patients were also comparable to those in the patients without cardiovascular disease (results not shown).

**Discussion**

In the present study, we have demonstrated that only a small portion of patients with severe hypertriglyceridemia have elevated complexes of factor XIIa and kallikrein with C1
Furthermore, lowering plasma triglycerides did not effect these activation markers. Because activation of the contact system, and especially FXII, by long-chain fatty acids is considered to be a potential mechanism for FVII activation and related cardiovascular risk in patients with hypertriglyceridemia, the present results are important for understanding the cardiovascular risk profile in these patients.

Baseline

Approximately 10% of the patients had evidence of contact activation; ie, in 13% and 9% of the patients, FXIIa– and kallikrein–C1 inhibitor complexes were above the normal values for these assays. However, plasma concentrations of these complexes were low. This result is in concordance with previous studies, demonstrating <0.05% of FXII activation in middle-aged men without clinical symptoms of atherosclerosis and in 21 men with combined hyperlipoproteinemia, suggesting the contact system to be slightly activated in nonacute situations in men with enhanced risk for vascular disease.25,26 Furthermore, the slightly increased FXII antigen levels compared with normal values (≈400 nmol/L) and the PK antigen levels comparable with given normal values (≈550 nmol/L) do not support a high level of activation of these contact factors.

We assessed activation of FXI by measuring FXIa in complex with C1 inhibitor or α1-antitrypsin, and the latter complex was detected in 71% of the patients. In particular, the elevation of FXIa–α1-antitrypsin complex in two thirds of the patients compared with the almost-undetectable levels of FXIa–C1 inhibitor complex is striking. Although C1 inhibitor is the predominant inhibitor of FXIa owing to a relatively long plasma half-life of inhibition, FXIa–α1-antitrypsin complexes may be a better parameter for establishing FXI activation in vivo.23,27 Moreover, the increased levels of FXIa–α1-antitrypsin complex in the presence of normal levels of FXIa–C1 inhibitor complex virtually exclude the possibility that these results were influenced by in vitro artifacts, since in that case, higher levels of FXIa–C1 inhibitor complexes would be expected.23 FXIa–α1-antitrypsin complexes have also been detected in patients with coronary artery disease and in patients with non–insulin-dependent diabetes mellitus,28,29 suggesting that these complexes are a sensitive marker for atherosclerotic vascular disease, although clinically evident vascular disease was present in only a small fraction of patients in our study. Enhanced activation of FXI was not reflected by a decrease in FXI antigen levels, which were comparable with normal values (≈31 nmol/L).

In the revised model of coagulation, FXI is activated by thrombin instead of FXII.30 In this study, we found a weak but significant correlation between FXIa–α1-antitrypsin complexes and TAT complexes, indicating that activation of these clotting factors might be related. In 2 recent studies, it was shown that cell-free phospholipids, like atherogenic lipoproteins, can support the assembly of the prothrombinase complex and thrombin generation in vitro.31,32 We speculate that in these hypercholesterolemic patients, thrombin generated on atherogenic phospholipids might be responsible for FXI activation and thereby, the increased values of the FXIa–α1-antitrypsin complex. However, because elevated markers of contact activation were also present, we cannot exclude the

<table>
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<th>TABLE 3. Markers of Thrombin Generation Before and After Treatment With n-3 PUFAs or Gemfibrozil</th>
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<tr>
<td>Before Treatment</td>
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<tr>
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<tr>
<td>TAT, µg/L</td>
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<td>F1+2, nmol/L</td>
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<td>F1+2, gemfibrozil, nmol/L</td>
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Median and range are given; statistical analysis (Wilcoxon rank test) for differences between levels before and after treatment were performed on both treatment groups together and for F1+2 for each treatment group separately.
The observation that changes in the activation markers FXII or PK did not occur, whereas triglyceride levels decreased with ±50%, suggests that triglyceride levels did not influence contact activation. Our results are in accordance with those of Broijersen et al., who also did not detect an effect of FXII on activation in the plasma of hypertriglyceridemic patients after treatment with gemfibrozil for 10 to 12 weeks. These combined results do not support the notion that FXII is activated by long-chain fatty acids present in triglycerides. Furthermore, not only in chronic hypertriglyceridemia but also after a high-fat meal are triglycerides elevated and positively associated with FVII-c. FXII could be excluded as a mediator in the postprandial activation of FVII by 2 recent studies, which demonstrated a clear FVIIa response after a high-fat meal in homozygous FXII-deficient patients. Moreover, in healthy individuals, no changes in FXIIa were measured after a high-fat meal.

We therefore conclude that the contact system is not activated by triglycerides in vivo. Contact activation is not likely to contribute to the hypercoagulability observed in hypertriglyceridemic patients. However, FXI activation is frequently present and is positively correlated with the level of TAT complexes.

**Acknowledgments**
M.C. Minnema was supported by a grant of the Netherlands Heart Foundation (No. 94024). The authors thank the members of the Laboratory of Special Hemostasis (AMC, G1) and Y.P.T. Lubbers from the Laboratory of Clinical and Experimental Immunology (CLB, U110) for their technical assistance.

**References**

**Effects of Treatment**
Patients were treated with gemfibrozil or n-3 PUFAs for a period of 12 weeks. The fibrate gemfibrozil is effective in lowering elevated plasma triglyceride and cholesterol levels due to the stimulation of lipoprotein lipase activity. The lipid-regulating effect of n-3 PUFAs is also established; however, the working mechanism for its effect has yet to be resolved.

After therapy, there was a highly significant decrease in triglyceride and cholesterol levels in both treatment groups. Levels of prothrombin F1+2 decreased, but this was only significant in the gemfibrozil group. Two other studies have demonstrated a decrease of F1+2 after treatment with gemfibrozil in patients with coronary heart disease or with combined hyperlipidemia. The lack of change in F1+2 in the n-3 PUFA treatment group is in contrast with a previous study, in which a significant decrease was detected in 20 patients after 16 weeks of therapy. In this study, however, only patients with chronic atherosclerosis and strongly elevated levels of F1+2 were included. The TAT complexes did not follow the decrease in F1+2 in the gemfibrozil group, possibly owing to a lower sensitivity of this assay. After therapy, no change in FXIa-α1-antitrypsin complexes was observed, and complexes remained significantly correlated with TAT complexes (Figure 3). The decrease in thrombin generation was most likely too small to cause any effect on FXI activation.

Surprisingly, each of the antigen levels of FXII, PK, and FXI increased in the gemfibrozil group only. Increases in zymogen concentration may be due to either increased synthesis, decreased catabolism, or both. Because antigen concentrations were in the normal range at the beginning of the study and we could not detect any change in activation markers, these increases most likely do not reflect a decrease in activation of the respective zymogens. An explanation could be the different mode of action of n-3 PUFAs and gemfibrozil or the possible interference of gemfibrozil therapy with the clearance of clotting factors from the circulation.
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doi: 10.1161/01.ATV.19.10.2548

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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