Effect of Alteration in Triglyceride Levels on Factor VII-Phospholipid Complexes in Plasma

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Clotting Factor VII activity emerges as a highly significant predictive factor for development of cardiovascular disease (CVD) in prospective trials. We have previously shown that in hypertriglyceridemic individuals a fraction of their clotting Factor VII molecules is present in an activated state in phospholipase C-sensitive complexes in plasma. These complexes may explain the increased Factor VII activity observed to be a predictive factor for CVD. We demonstrate here that the level of such complexes is amenable to dietary intervention and that the alteration in Factor VII complex levels correlates closely with the corresponding alteration in triglycerides.


There is growing evidence of a role for increased plasma triglycerides in atherogenesis. Recently, hypertriglyceridemia has emerged as a significant procoagulant state associated with increased t-PA inhibitor levels, activated Factor VII-phospholipid complexes, and increased Factor X clotting activity.

We have previously reported the presence of increased levels of Factor VII-phospholipid complexes in men at risk for cardiovascular disease. Previous reports by Meade et al., Miller et al., and recently by Balleisen et al. have shown that Factor VII clotting activity is a predictive factor for development of coronary heart disease. The potential importance of the Factor VII complexes in risk prediction is currently under investigation in two prospective studies.

Here we address the question of whether the level of such complexes is amenable to dietary intervention and whether their variations correlate with observed variations in triglyceride levels.

Methods

Subjects

Forty-one healthy men 40 years old were drawn from the high-risk group in the Oslo Study, a randomized, controlled, prospective study of the effect of intervention in lifestyle (diet, smoking, physical exercise) in Norwegian men. High risk was defined according to the risk score used in that study, i.e., based on cholesterol levels, smoking, and systolic blood pressure as detailed elsewhere. Selection for the study occurred before triglycerides and Factor VII levels had been measured. Each individual was investigated when entering the study and again 3 months later.

Blood Sampling and Testing

After informed consent, fasting venous blood was obtained before 9 A.M. by using triple siliconated Vacutainers containing 0.129 M sodium citrate (Becton-Dickinson, Plymouth, U.K.). Blood samples were centrifuged at 10,000 g for 10 minutes at 20°C to obtain platelet-poor plasma. These samples were kept at room temperature to avoid cold activation of Factor VII. We have previously shown that the level of total Factor VII activity and the level of Factor VII-phospholipid complex remain stable for at least 4 hours with this procedure.

The total Factor VII activity was assayed in triplicate in a one-stage system, in which human brain thromboplastin and plasma from a patient congenitally deficient in Factor VII were used as substrate. The plasma sample to be tested was incubated for 10 minutes at 37°C so it would be handled in exactly the same way as the phospholipase C (PLC)-treated sample. Standard curves were established by dilution of “Reference Plasma” 100% (Lot No R51, Immuno, Vienna, Austria). The mean Factor VII activity of 48 healthy men with a mean age of 42 years who were at low risk for coronary disease (score <5, serum triglycerides <1.5 mmol/l) was 87 units/ml. This was taken to represent 100% activity.

Other Methods

PLC was purified to homogeneity from Bacillus cereus culture supernatants according to the method of Little et al., as modified. PLC purified according to the method of Myrnes and Little was kindly donated by Professor Clive Little, University of Tromsø, Tromsø, Norway. The two preparations had the same specific activity. Residual Factor VII activity after PLC treatment was assayed by incubating plasma with PLC (final concentration, 5 μg/ml) for 10 minutes at 37°C and subsequently testing the plasma in the Factor VII assay system. The presence of PLC did not influence the test system. The level of

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phospholipase C-sensitive Factor VII complex was calculated by subtracting this residual activity of Factor VII from the total Factor VII activity.

Serum total cholesterol was measured with an enzymatic colorimetric method (CHOD-PAP), and high density lipoprotein (HDL) cholesterol was similarly measured after precipitation with phosphotungstic acid. Triglycerides were determined by enzymatic hydrolysis and subsequent measurement of the liberated glycerol, by colorimetry (GPO-PAP). All kits were provided by Boehringer Mannheim, Mannheim, FRG.

**Dietary Intervention**

The intervention consisted of dietary changes only, including adjustment of calorie intake to achieve and maintain optimal body weight, restriction of total fat intake to a maximum of 30% of total calories consumed, and a polyunsaturated/saturated (P/S) fatty acid ratio of 1.0. No drugs were used. The standard Norwegian diet consists of about 37% of calories from fat, with a P/S ratio of about 0.4. Sugar contributes about 14% of calories.

Dietary advice included substitution of polyunsaturated for saturated fats, mainly by using highly polyunsaturated margarine; use of polyunsaturated oils for cooking; increasing the intake of fatty fish; and reducing the intake of high fat dairy products, such as whole milk, cheese, and butter. Reduction of concentrated sweets, chocolate, soft drinks, and alcoholic beverages was recommended. Reduced intake of complex carbohydrates and starch was not suggested; in many cases an increase was recommended, e.g., by increased intake of fiber-rich bread.

**Results**

The results of the dietary intervention on blood lipids and Factor VII complex is given in Table 1. Total cholesterol was reduced by 0.86 mmol/l (11%) without significant reduction of HDL cholesterol. Triglycerides were reduced by 0.73 mmol/l (27%). Total Factor VII did not change significantly, but the level of PLC-sensitive Factor VII complexes was reduced by about one fourth, from 35% to 26%.

To demonstrate more clearly the correlation between the levels of triglyceride and Factor VII complex, the differences between pre- and post-intervention values for triglycerides (Δtriglycerides) were plotted against the corresponding differences for Factor VII complex (ΔF.VII) for each individual (Figure 1), and the linear regression was calculated. In the 25 cases where dietary intervention had the desired effect, as evidenced by a reduction in triglyceride levels, the level of Factor VII complex also declined in 22 cases. In the cases of 14 of 41 men, their triglyceride levels actually increased (plotted below the abscissa in Figure 1). In 11 of these men, their levels of Factor VII complex also increased. Two men with minor increases in triglycerides (0.20 and 0.38 mmol/l) had essentially unaltered Factor VII levels. The overall correlation coefficient was 0.87, 0 < 0.0005.

The correlation coefficient for the difference between pre- and post-intervention total cholesterol values (Δcholesterol and ΔF.VII) was 0.67 (p < 0.0001). No significant correlation with ΔHDL cholesterol was found. The multiple regression coefficient for ΔF.VII versus Δtriglycerides and Δcholesterol was 0.87; i.e., the inclusion of Δcholesterol in the equation did not alter the correlation coefficient.

**Discussion**

Clotting Factor VII is activated several-fold when present in the PLC-sensitive complex state in plasma. This activation is reversible in vitro when such complexes are treated with PLC and is thus probably not caused by nicking or other permanent changes to the Factor VII protein.

The level of Factor VII activity in plasma is of considerable interest as a potential predictive risk factor for cardiovascular disease, as first shown by Meade et al. In a subsample of men who had developed cardiovascular disease, these researchers demonstrated that the level of Factor VII antigen was normal, albeit the clotting activity...
was increased. Data confirming a role for Factor VII as a risk factor have been reported briefly by Balleisen et al.\textsuperscript{12} Pending further confirmation, the PLC-sensitive Factor VII complexes are very good candidates for a role as a risk factor. We have previously shown a close correlation between the levels of Factor VII complex and triglycerides in plasma.\textsuperscript{6}

In the present article, we have shown that the level of such complexes in plasma is amenable to short-term (3-month) dietary intervention; there is a close correlation between the difference in triglyceride levels before and after intervention and the corresponding levels of Factor VII complex. Thus, whenever the dietary intervention was effective (i.e., had a triglyceride-lowering effect), a lowering of Factor VII complex values was also observed. The clotting factor activity increase is obviously reversible in vivo. Its close correlation with triglyceride level is further evidence that the previously observed association between these two parameters was not fortuitous.

Although mean reductions of cholesterol (11%) and triglycerides (28%) were seen, the intervention was not successful in all cases. Fourteen out of 41 individuals actually had increased fasting triglyceride levels (Figure 1). This was mainly because, in many cases, more than one lifestyle change was required to induce dietary and other lifestyle changes. The study did, however, enable us to demonstrate that the covariation of triglyceride and Factor VII complex levels holds for increasing, as well as for decreasing, triglyceride levels, and this was the question we wanted to answer. Although not the primary goal of this investigation, the intervention did bring mean total Factor VII activity down to a level very close to that in the low-risk population investigated. In that population, however, the level of complex was negligible.

Our data show that Factor VII correlates more closely with $\Delta$triglyceride levels than with $\Delta$total cholesterol, and the multiple regression coefficient for $\Delta$F.VII versus $\Delta$triglycerides and $\Delta$cholesterol was not different from the correlation coefficient for $\Delta$triglycerides alone, suggesting that the apparent association of changes in Factor VII complexes and cholesterol can be explained indirectly by the association of $\Delta$cholesterol with triglycerides. Thus, altered levels of cholesterol may not independently influence the level of PLC-sensitive Factor VII complexes.

In conclusion, hypertriglyceridermia emerges as a state where the hemostatic balance is altered in the direction of thrombosis by this increase of Factor VII activity in addition to the increase in Factor X\textsuperscript{7} and the reduction in fibrinolysis.\textsuperscript{3,20} At least two of these factors, the increased Factor VII activity and the reduced fibrinolytic potential, can be altered by an intervention that alters the triglyceride levels.\textsuperscript{11,20}

The mechanism(s) whereby the PLC-sensitive Factor VII complexes are formed remain unknown. Similar complexes are seen in the plasma of pregnant women\textsuperscript{21} but disappear shortly after the birth of the placenta. In gel filtration experiments, these complexes move with a $K_0$ that is essentially the same as that of the complexes derived from plasma samples from men. Although the complexes thus have about the same molecular radii, this does not mean that they are formed by the same mechanism.

Whether the complexes in either case arise by spontaneous transfer of phospholipids or by some sort of facilitated process (enzymatic or by a transfer protein) is totally unknown. The Factor VII molecule has some short, highly hydrophobic sequences that might form complexes with the hydrophobic part of phospholipids, but so have many other proteins not known to form such complexes. Until now, formation of similar complexes in vitro has, in our hands, been unsuccessful, but the experimental possibilities are far from exhausted.

Preliminary studies in dogs (Spurling and Prydz, unpublished observation) suggest that a similar complex can be formed very rapidly, i.e., in the postprandial period after a fatty meal. This animal model may allow more detailed studies of the nature and formation of these complexes.

References


Index Terms: triglyceridemia • factor VII • cardiovascular disease