Correlation of Vitamin K-Dependent Clotting Factors With Cholesterol and Triglycerides in Healthy Young Adults

Carol J. Hoffman, William E. Lawson, Robin H. Miller, Mae B. Hultin

Abstract The plasma level of factor VII activity was a risk factor for the development of ischemic heart disease (IHD) in a prospective epidemiological study of hemostatic factors. We have previously reported significant correlations between factor VII clotting activity or antigen and lipids in a group of 132 young men (<30 years old) at low risk for IHD and concluded that control of the plasma factor VII level may be linked to lipid metabolism in normal male physiology. Because factor VII is one of four vitamin K-dependent procoagulant proteins, we hypothesized that plasma levels of all these proteins would be similarly controlled in normal physiology. In an extension of this study, we have measured two additional vitamin K-dependent clotting factors (prothrombin [factor II] and factor X activity), as well as factor VII activity and antigen and fasting serum lipid fractions in healthy young men and women (<30 years old) at low risk for IHD. In the women, we found significant positive correlations of factor VII antigen with total or HDL cholesterol and of prothrombin or factor X with total or LDL cholesterol. In the men, factor VII activity or antigen correlated with total cholesterol, triglycerides, HDL cholesterol, or LDL cholesterol; prothrombin or factor X correlated with total cholesterol, triglycerides, or LDL cholesterol. In contrast, we found no significant correlations of fibrinogen with any of the lipid fractions in our groups of men or women. Our data support the hypothesis that control of the levels of the vitamin K-dependent procoagulant proteins is linked to lipid metabolism in the normal physiology of both men and women.

Key Words • factor VII • triglycerides • cholesterol • prothrombin • factor X

The Northwick Park Heart Study identified elevated plasma factor VII clotting activity as a predictive risk factor for ischemic heart disease (IHD) in a prospective study of 1511 middle-aged men. Other investigators have reported a positive correlation between factor VII levels and total serum cholesterol, triglycerides, very-low-density lipoproteins (VLDL), and the large, triglyceride-rich lipoproteins, especially very-low-density lipoproteins (VLDL). The correlations of factor VII activity with lipid fractions in these studies may have reflected pathophysiologic changes related to the presence of silent or subclinical arteriosclerosis in the middle-aged and older subjects in the studies. We have previously demonstrated that young adults (mean age, 35 years) at high risk for IHD had significantly higher plasma factor VII activity and antigen levels than comparable young adults at low risk. We subsequently found that a similar group of high-risk young adults (mean age, 34.8 years) also had significant elevations of prothrombin, factor IX, and factor X clotting activities, as well as factor VII, compared with young adults at low risk. We have also found a strong positive correlation of factor VII activity and antigen with fasting levels of total serum cholesterol, serum triglycerides, high-density lipoprotein cholesterol (HDL-C), or low-density lipoprotein cholesterol (LDL-C) in a group of 132 younger adult men (<30 years old) at low risk for IHD. We concluded that control of the plasma factor VII level may be linked to lipid metabolism in the normal physiology of males. However, we did not measure levels of any of the other vitamin K-dependent clotting factors (factors II, IX, and X) in that study.

The purpose of the present report was to study the relation of several vitamin K-dependent procoagulant proteins (factor VII, prothrombin, and factor X) with lipid levels in a group of young adult men and women (<30 years old) at low risk of IHD to assess whether control of the levels of vitamin K-dependent procoagulant proteins is consistently linked to lipid metabolism in normal physiology.

Methods

The protocol for obtaining blood samples was approved by the Committee on Research Involving Human Subjects at the State University of New York at Stony Brook according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Blood samples were collected by venipuncture from subjects by a double-syringe technique after an overnight fast. Serum was prepared from the contents of the first syringe by centrifugation at 3000g for 20 minutes at 4°C within 1 hour of blood collection as previously reported. All samples were kept at room temperature before and after centrifugation to avoid cold activation. Plasma was divided into small (<1 mL) aliquots, immediately frozen, stored at -80°C, and assayed for factor VII, fibrinogen, prothrombin, or factor X within 1 month.
Correlation of prothrombin with total serum cholesterol levels in women. N=41, p=0.623. Regression equation: prothrombin = 56.3 + 0.299 × cholesterol (solid line); dotted lines represent 95% confidence interval around regression.

Young men (n=216, mean age, 23.3±3.2 years) and women (n=81, mean age, 23.7±3.7 years) from incoming freshmen medical school classes were recruited as volunteer subjects over a 5-year period. Women currently on oral contraceptive therapy were excluded from this study, as this medication has been shown to cause a significant increase in factor VII, fibrinogen, and triglycerides. None of the subjects for this study was on any other medication. Subjects with a family history of premature IHD (evidence of IHD before age 50 in men or before age 60 in women) were also excluded.

Assays

Serum total cholesterol and triglyceride levels were assayed by DART reagents on a DACOS analyzer (Coulter Diagnostics) according to the manufacturer’s directions. Serum HDL was isolated by a phosphotungstic acid precipitation procedure (Data Medical Associates, Inc.), and HDL-C was assayed on a DACOS analyzer. Serum LDL-C was calculated by the Friedewald equation.

Factor VII clotting assays and factor VII enzyme immunoassays were performed as previously described, predominantly in the first 4 years of the study. Fibrinogen and prothrombin clotting assays were also performed as previously reported, during the last 3 years of the study, and factor X assays in the last 2 years. Therefore, there were a different number of subjects for each of these assays. Factor X clotting activity was assayed by a modified method of Jesty and Nemerson. A stock solution of Russell’s viper venom (RVV, Sigma Chemical Co) was prepared as described, and diluted 1:100 in Tris-buffered saline/bovine serum albumin (TBS/BSA) (0.1 mol/L NaCl [Fisher]/0.05 mol/L Tris [Sigma]/0.1% [wv] BSA fraction V, fatty-acid free, Sigma), pH 7.5, for a final concentration of 0.002 mg/mL RVV (RVV/10). One hundred microliters of test sample was incubated with 0.1 mL of human factor X-deficient plasma (George King Bio-Medical) containing 1/10 volume of 20 mg/mL soy lecithin (Central Soya) at 37°C for 3 minutes. Then 0.1 mL of 37°C RVV/100 was added. Thirty seconds later, 0.1 mL of 25 mmol/L CaCl2 at 37°C was added, and the clotting time was determined on a Fibrometer (BBL). The standard curve was prepared by assaying pooled normal plasma at 1/5, 1/10, 1/20, 1/50, and 1/100 dilutions in TBS/BSA. Control and subject plasma samples were assayed at 1/20, 1/10, and 1/5 dilutions. The interassay coefficient of variation was 4%, based on 14 determinations of a single normal donor’s plasma.

Reference pooled normal plasma (PNP) was prepared in our laboratory from healthy male and female volunteers and was arbitrarily assumed to have 100% factor VII and prothrombin activity. A fibrinogen calibration reference standard (Organon Teknika) was used for the fibrinogen assays. PNP was prepared and stored under the same conditions as those used for the subject samples of this study. Three subsequent batches of PNP were also prepared in our laboratory from healthy male and female donors. Each batch was calibrated against the previous one before use in the assays by the simultaneous assay method of North et al. Factor X assays were first determined using batch 3 of our pooled normal plasma, which was arbitrarily assumed to have 100% factor X activity. The subsequent batch of PNP (batch 4) was calibrated against batch 3 before use in the assay. In addition, a single normal donor’s plasma was assayed with each set of factor VII, fibrinogen, prothrombin, or factor X assays to serve as a daily internal control. The same normal donor was used in all assays over the entire course of this study to further serve as an internal reference.

Statistics

Results of fibrinogen, prothrombin, factor X, factor VII clotting, and factor VII enzyme immunoassays were analyzed on a personal computer (Dell System 200) by logarithmic transformation of the data in the PARLIN program for parallel-line analysis of bioassays, as previously described. All other analyses were performed on a personal computer (BSR, 386-SX, Duk) using CSS and STATISTICA softwares (Statsoft). Observed distributions of all clotting factor and lipid assays were analyzed for departure from expected values of the normal distribution by chi² and Kolmogorov-Smirnov d statistics. Be-

**TABLE 1. Plasma Factor VII, Fibrinogen, Prothrombin, and Factor X Levels and Serum Lipid Levels in Young Men and Women**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
<td></td>
<td>Mean±SD</td>
<td>Median</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>FVIIc, %</td>
<td>95±21</td>
<td>94</td>
<td>43-160</td>
<td></td>
<td>93±18</td>
<td>90</td>
<td>61-148</td>
<td></td>
</tr>
<tr>
<td>FVII-ag, %</td>
<td>91±22</td>
<td>88</td>
<td>43-167</td>
<td></td>
<td>86±18</td>
<td>86</td>
<td>55-129</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>234±54</td>
<td>227</td>
<td>146-507</td>
<td></td>
<td>246±50</td>
<td>233</td>
<td>153-385</td>
<td></td>
</tr>
<tr>
<td>Prothrombin, %</td>
<td>101±18</td>
<td>102</td>
<td>58-138</td>
<td></td>
<td>104±14</td>
<td>103</td>
<td>73-131</td>
<td></td>
</tr>
<tr>
<td>FX, %</td>
<td>100±17</td>
<td>99</td>
<td>68-154</td>
<td></td>
<td>94±21</td>
<td>95</td>
<td>60-147</td>
<td></td>
</tr>
<tr>
<td>Total chol, mg/dL*</td>
<td>171±36</td>
<td>165</td>
<td>97-338</td>
<td></td>
<td>163±25</td>
<td>160</td>
<td>120-226</td>
<td></td>
</tr>
<tr>
<td>Trig, mg/dL*</td>
<td>84±50</td>
<td>73</td>
<td>17-360</td>
<td></td>
<td>60±30</td>
<td>55</td>
<td>23-189</td>
<td></td>
</tr>
<tr>
<td>HDL-C, mg/dL*</td>
<td>52±16</td>
<td>49</td>
<td>20-111</td>
<td></td>
<td>63±18</td>
<td>60</td>
<td>35-126</td>
<td></td>
</tr>
<tr>
<td>LDL-C, mg/dL*</td>
<td>102±34</td>
<td>99</td>
<td>31-280</td>
<td></td>
<td>88±26</td>
<td>81</td>
<td>46-166</td>
<td></td>
</tr>
</tbody>
</table>

FVIIc indicates factor VII clotting activity; FVII-ag, factor VII antigen; FX, factor X clotting activity; Total chol, total serum cholesterol; Trig, triglycerides; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

*All lipid levels were measured on blood obtained after an overnight fast.
TABLE 2. Correlation of Clotting Factor Assays With Fasting Lipid Levels in Young Men

<table>
<thead>
<tr>
<th>Total Serum Cholesterol</th>
<th>Triglycerides</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIIc, n=165 p (P)</td>
<td>.349 (4.0x10^-7)</td>
<td>.161 (.039)</td>
<td>.181 (.02)</td>
</tr>
<tr>
<td>FVII-ag, n=172 p (P)</td>
<td>.445 (9.28x10^-7)</td>
<td>.247 (.001)</td>
<td>218 (.004)</td>
</tr>
<tr>
<td>Pro, n=104 p (P)</td>
<td>.295 (.002)</td>
<td>.268 (.006)</td>
<td>-.192 (NS)</td>
</tr>
<tr>
<td>FX, n=69 p (P)</td>
<td>.499 (1.3x10^-6)</td>
<td>.397 (.0007)</td>
<td>-.205 (NS)</td>
</tr>
</tbody>
</table>

FVIIc indicates factor VII clotting activity; FVII-ag, factor VII antigen; Pro, prothrombin clotting activity; FX, factor X clotting activity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; p, Spearman correlation coefficient; and NS, not significant.

cause the observed lipid values were not normally distributed, correlations of factor VII, fibrinogen, prothrombin, or factor X assays with serum triglycerides, total serum cholesterol, HDL cholesterol, or LDL cholesterol were analyzed by Spearman's p statistic, a nonparametric statistic of rank-order correlation. A probability value of P<.05 was considered significant. Differences between HDL-C levels or triglyceride levels in our male versus female subjects were tested for significance by the Mann-Whitney U test (CSS: STATISTICA software).

Results

Results of factor VII, fibrinogen, prothrombin, factor X, and lipid assays are summarized in Table 1. Median triglyceride levels were significantly higher in men than in women (P=2.08x10^-7), and median HDL-C levels were lower in men than in women (P=6.64x10^-10). Seven men had a total cholesterol level >240 mg/dL, and six men had a triglyceride level >200 mg/dL. In contrast, no female subjects had a total cholesterol level >240 mg/dL or a triglyceride level >200 mg/dL.

Table 2 summarizes correlations of clotting factor assays with fasting lipid levels in our male subjects. There were significant correlations of factor VII activity and antigen with total cholesterol, triglycerides, HDL-C, and LDL-C. Prothrombin activity and factor X activity correlated significantly with total cholesterol, triglycerides, and LDL-C but not HDL-C. These correlations remained highly significant when the 13 men with total cholesterol levels >240 mg/dL or fasting triglyceride levels >200 mg/dL were excluded, with the exception of the correlation of factor VII clotting activity and triglycerides (n=153, P=.17). There were no significant correlations between fibrinogen and any of the lipid fractions in these men (n=137, P>.05).

Table 3 summarizes the correlations of clotting factor assays with lipid levels in our female subjects. There were no significant correlations between factor VII clotting activity with any of the lipid levels in these women (P>.05). Factor VII antigen correlated significantly with total cholesterol and HDL-C, while prothrombin clotting activity and factor X clotting activity correlated significantly with total cholesterol and LDL-C. A graphic representation of the correlation of prothrombin with total cholesterol levels in women is shown in the Figure. In contrast, there were no significant correlations between fibrinogen levels and any of the lipid fractions in these women (n=45, P>.05).

Discussion

Other investigators have reported correlations between dietary fat intake and factor VII coagulant activity in males. Positive correlations between factor VII activity or antigen and triglycerides, total cholesterol, and HDL cholesterol have been consistently reported in healthy or hyperlipidemic men of all ages. Fewer data are available on the relation of factor VII to lipid levels in women, especially young healthy women. Some studies have included hyperlipidemic or healthy middle-aged and elderly women but have not analyzed them separately. One large study of healthy women (mean age, 37 years) found positive correlations of factor VII clotting activity with triglycerides, cholesterol, and HDL cholesterol. Factor VIIa levels were also reported to be positively correlated with HDL cholesterol in men and women. All of the aforementioned correlations were significant (P<.05 to P<.001) by univariate and/or multivariate analysis. All investigators either used nonparametric statistics or performed analyses on logarithmically transformed values because of the non gaussian distribution of most of the variables. Factor VII, prothrombin, and factor X can bind to the triglyceride-rich very-low-density lipoproteins, perhaps by hydrophobic interactions. These interactions may be part of the mechanism by which lipoproteins influence the levels of vitamin K-independent coagulant proteins.

We found strong correlations between factor VII clotting activity and serum lipid fractions in our sample of healthy young men. In contrast, we found no correlations between factor VII clotting activity and any of the serum lipid fractions measured in our sample of healthy young women. The usefulness of factor VII

TABLE 3. Correlation of Clotting Factor Assays With Fasting Lipid Levels in Young Women

<table>
<thead>
<tr>
<th>Total Serum Cholesterol</th>
<th>Triglycerides</th>
<th>HDL-C</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIIc, n=58 p (P)</td>
<td>.189 (NS)</td>
<td>-.001 (NS)</td>
<td>.196 (NS)</td>
</tr>
<tr>
<td>FVII-ag, n=60 p (P)</td>
<td>.310 (.016)</td>
<td>.058 (NS)</td>
<td>.281 (.030)</td>
</tr>
<tr>
<td>Pro, n=41 p (P)</td>
<td>.623 (1.4x10^-6)</td>
<td>.275 (NS)</td>
<td>-.079 (NS)</td>
</tr>
<tr>
<td>FX, n=30 p (P)</td>
<td>.730 (5.0x10^-9)</td>
<td>.145 (NS)</td>
<td>-.117 (NS)</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FVIIc, factor VII clotting activity; p, Spearman correlation coefficient; NS, not significant; Full-ag, factor VII antigen; Pro, prothrombin clotting activity; and FX, factor X clotting activity.
clotting activity as a predictive risk factor in men may be dependent on this gender-associated specific correlation between factor VII clotting activity and serum lipids. Although we did find significant correlations between factor VII antigen levels and total cholesterol or HDL-C in our female subjects, as have other investigators, the correlations were much stronger statistically in the men. We also found significant correlations between prothrombin levels or factor X levels and serum lipid fractions in both men and women. Our sample of healthy young men and women appears to be representative of a normal population at low risk of IHD, because <5% of the sample had fasting total serum cholesterol levels >240 mg/dL or triglycerides >200 mg/dL. The factor VII antigen and activity levels, as well as fibrinogen levels, were in the expected range for subjects in the third decade of life.

The lack of correlation between fibrinogen and any lipid fractions in our sample of healthy young men and women suggests that the significant correlations we found between the vitamin K–dependent clotting factors and lipid fractions in these groups were not due to a general or nonspecific effect on protein metabolism. These data support the hypothesis that control of the plasma levels of the vitamin K–dependent proteins as a group is closely linked to lipid metabolism in the normal physiology of both men and women.

Acknowledgments

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References

Correlation of vitamin K-dependent clotting factors with cholesterol and triglycerides in healthy young adults.

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