An Association Between Clotting Factor VII and Carotid Intima-Media Thickness
The CARDIA Study

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Background and Purpose—To investigate associations of procoagulants (factor VII [FVII], FVIII, von Willebrand factor) with subclinical atherosclerosis, we examined participants in the Coronary Artery Risk Development in Young Adults (CARDIA) study.

Methods—Clotting factor assays were performed in 1254 participants 23 to 37 years of age (baseline) and repeated at ages 38 through 50 (follow-up). Carotid intima-media thickness (IMT) was measured at follow-up.

Results—Baseline levels of procoagulants (%), mean (SD) were: FVII, 76 (18); FVIII, 102 (38); and von Willebrand factor, 108 (47). At follow-up, all had increased by 40% to 55%. After age adjustment, mean common carotid IMT increased from the lowest to the highest tertile of FVII in the total group (0.787 to 0.801; P=0.007), in whites (0.772 to 0.790; P=0.002), and in men (0.807 to 0.827; P=0.015). All associations were attenuated by multivariable adjustment. However, participants with FVII values in the highest tertile at one or both examinations, compared with those in the lowest tertile, had greater common carotid IMT after age and multivariable adjustment (0.806 versus 0.778; P<0.05). Baseline FVIII was associated with greater internal carotid IMT in the total group, in whites, and in women after age adjustment but not multivariable adjustment. No associations were seen for von Willebrand factor.

Conclusions—FVII is associated with common carotid IMT in young adults, but the strength of the association is modified by other cardiovascular disease risk factors, such as body mass index. FVIII is associated with internal carotid IMT only in age-adjusted analyses, and no associations were observed for von Willebrand factor. (Stroke. 2010;41:1417-1422.)

Key Words: factor VII ■ carotid thickening ■ atherosclerosis ■ factor VIII

Ischemic stroke is characterized by thrombotic occlusion of cerebral vessels, but whether elevated concentrations of specific coagulant proteins are associated with stroke risk is unclear. The question is important because if such relationships were established, measuring procoagulant protein concentrations might provide prognostic as well as therapeutic approaches to stroke management. To identify associations of procoagulants with the development of vascular disease, we measured hemostatic factors in healthy young adults, 25 to 37 years of age. Thirteen years later, we repeated the hemostatic factor measurements and searched for evidence of subclinical atherosclerosis. We then determined whether there were associations between the clotting factor levels at either time interval and the presence of subclinical cardiovascular disease (CVD).

Methods
Coronary Artery Risk Development in Young Adults Study
Participants were from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a multicenter longitudinal study designed to investigate the evolution of CVD risk factors and subclinical atherosclerosis. Details of the design have been published previously. All data collection technicians were centrally trained and certified, and the CARDIA coordinating center and the CARDIA quality control committee monitored data collection throughout the study. The study was approved by the institutional review board at each field center, and informed consent was obtained from each participant at each examination. Participants’ age, race, gender, and cigarette use were assessed by questionnaire. Anthropometric variables included height and weight, body mass index (BMI), and blood pressure. Height and weight were measured using a balance beam scale and a vertical ruler, respectively, with the participant wearing light clothing and no shoes. BMI was calculated as the weight (kg) divided by the height in meters squared (m²). The resting blood pressure was measured in the right arm using a random-zero sphygmomanometer at baseline and with an automated Omron device at follow-up. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or current use of antihypertensive medication. Biochemical variables included total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein cholesterol, triglycerides, C-reactive protein (CRP), fasting glucose, and insulin. Diabetes was defined as fasting glucose ≥7.0 mmol/L or current use of diabetic medications. In 1992 and 1993, as part of the year-7 CARDIA examination, hemostatic...
factors were measured along with other clinical, demographic, and health variables in 2 of the 4 CARDIA sites (Chicago and Minneapolis). Hemostatic factors were again measured during the year-20 CARDIA examination in 2005 and 2006. We refer to the Y7 and Y20 examinations as the baseline and follow-up examinations throughout this article.

Figure 1 is a flowchart showing the initial number of participants, the number analyzed at baseline and follow-up, and the reasons for exclusion. According to baseline data, persons who had missing data or who were lost to follow-up were more likely to be black, male, younger, less educated, and smokers than participants in the study sample.

Data Collection
Carotid ultrasound was performed at the follow-up examination by trained technicians at each field center using a Logiq 700 ultrasound machine (General Electric Medical Systems). The procedures were performed in the supine position with the participant’s head rotated 45° away from the side of the study. Magnified gray-scale images of the bilateral far and near wall of the distal common carotid (CC), the bulb, and the proximal internal carotid (IC) arteries were obtained. The maximum intima-media thickness (IMT) of the CC (CC-IMT) and the bulb/IC (IC-IMT) were defined as the mean of the maximum IMT of the near and far wall on both the left and right sides. Measurements were made at a central reading center by readers blinded to all clinical information. The number of measurements that were available for averaging ranged from 1 to 4 for the CC and 1 to 16 for the bulb/IC. The intrarater reproducibility of this method (Spearman correlations) is 0.75 for the CC-IMT and 0.86 for the IC-IMT.

At baseline, factor VII (FVII) and FVIII coagulant activities were assayed in citrate plasma as we have described previously. At follow-up, FVII antigen was assayed in EDTA plasma using the Aserachrom VII-Ag reagents and ELISA obtained from Diagnostica Stago. FVIII antigen was assayed in EDTA plasma using the VisuLize FVIII Antigen Kit (Affinity Biologicals), which uses a double-antibody ELISA. Von Willebrand factor (vWF) antigen was measured in EDTA plasma by an immunoturbidimetric method (vWFag; latest vWF Antigen Kit; Diagnostica Stago).

The coefficients of variation of the assays were determined by using split samples derived from the study participants. At baseline, the coefficients of variation for FVII and FVIII were 13% to 14%, and for vWF, 17%. At follow-up, the coefficient of variation for FVII was 5.1%, FVIII, 5.2%, and vWF, 6.0%. The smaller coefficients of variation for the assays at follow-up reflect the greater precision of the antigen assays compared with the clotting assays. Collection of samples in liquid citrate at baseline but in solid EDTA at follow-up introduces a dilution factor that decreases the values at baseline by approximately 10% compared with follow-up.

Statistical Analyses
Because of potentially large differences in associations of IMT with individual hemostatic factor by gender and race, all analyses were performed separately for individual hemostatic factor stratified by gender and race. First, baseline characteristics were computed for the participants, and differences between black and white within gender were assessed by either t tests for continuous variables or χ² tests for categorical variables. Next, a series of models was fit, with CC-IMT and IC-IMT at follow-up, as the outcomes. Linear regression models were used to examine the cross-sectional association at follow-up between IMT and hemostatic levels (based on tertiles of individual hemostatic factor within the entire cohort) after adjustment for follow-up age, sex, race (black or not), BMI, smoking (yes/no), education (in years), center (Chicago/Minneapolis), systolic blood pressure, diabetes (yes/no), antihypertensive medication use (yes/no), total and HDL cholesterol, and CRP (the log-transformed value was used to minimize the skewness of the distribution). Linear regression models were also used to evaluate the longitudinal associations between baseline hemostatic levels (based on tertiles of individual hemostatic factor within the entire cohort) and hemostatic factor groups (as defined below) with follow-up IMT, adjusted for baseline age, sex, race (black or not), BMI, smoking (yes/no), education (in years), center (Chicago/Minneapolis), systolic blood pressure, diabetes (yes/no), antihypertensive medication use (yes/no), total and HDL cholesterol, and log-transformed CRP. The final models for the change in hemostatic factor tertile group analyses also included baseline fibrinogen as a covariate. To test for linear trend, we included hemostatic factor as a continuous variable in multivariable models using linear regression for continuous outcomes (IMT). Multivariable adjusted logistic regression was also used to test differences between the hemostatic factor reference group (low-low) and other hemostatic factor groups (represented by the coefficient for the dummy variable of that group). To maximize the amount of information used in each analysis, we used all available data for each outcome (longitudinal, n = 1120 for CC-IMT and n = 1081 for IC-IMT; cross-sectional, n = 990 for CC-IMT and n = 961 for IC-IMT).

Associations of Hemostatic Groups With Subclinical Disease
To examine the associations of changes in each hemostatic factor over 13 years with subclinical CVD, participants were classified into 4 mutually exclusive groups based on the tertiles of individual factor (low, middle, high) at baseline and follow-up: group 1, those who were in the lowest tertile at baseline and follow-up (low-low); group 2, those who were in the lowest or middle tertile at baseline and follow-up (low-middle, low-middle, low-middle); group 3, those who were in the highest tertile at baseline and in the lowest or middle tertile at follow-up (high-low, high-middle) or those who were in the lowest or middle tertile at baseline and in the highest tertile at follow-up (low-high, middle-high); and group 4, those who were in the highest tertile at baseline and follow-up (high-high).

Managing Missing Data
To assess sensitivity because of missing information in some covariates and outcomes of interest, we performed analyses using multiple imputation. Although the missing proportion was ~23% of the original sample (1619; Figure 1), the imputed data sets resulted in consistent point and interval estimates compared with the estimates from unimputed data sets (data sets that exclude people who have partially observed information). The implementation of multiple imputation in this article was based on the procedure described by Raghunathan et al using IVEware software, and the standard errors adjusted for imputation were estimated using the SAS MINANALYZE procedure.
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Table 1. Characteristics of Study Participants According to Gender–Race Group, the CARDIA Study, 1992–2006*

<table>
<thead>
<tr>
<th></th>
<th>Total n=1254</th>
<th>Men (n=577)</th>
<th>Women, n=677</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black (n=189)</td>
<td>White (n=388)</td>
<td>Black (n=278)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>32.4 (3.5)</td>
<td>31.6 (3.7)†</td>
<td>32.6 (3.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.5 (5.7)</td>
<td>26.8 (5.3)</td>
<td>26.2 (4.1)</td>
</tr>
<tr>
<td>Education (y)</td>
<td>14.6 (2.5)</td>
<td>13.2 (2.2)†</td>
<td>15.4 (2.6)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>28.4</td>
<td>43.9†</td>
<td>20.4</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>1.2</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>108 (11)</td>
<td>112 (10)‡</td>
<td>110 (10)</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>1.3</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)§</td>
<td>177 (33)</td>
<td>177 (36)</td>
<td>180 (36)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)§</td>
<td>52 (14)</td>
<td>52 (15)†</td>
<td>45 (11)</td>
</tr>
<tr>
<td>CRP (µg/mL), median (IQR)</td>
<td>1.01 (0.45 to 2.78)</td>
<td>1.14 (0.51 to 2.55)†</td>
<td>0.77 (0.38 to 1.84)</td>
</tr>
<tr>
<td>FVII (%)</td>
<td>76 (18)</td>
<td>74 (18)</td>
<td>75 (18)</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>102 (38)</td>
<td>111 (44)‡</td>
<td>93 (30)</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>108 (47)</td>
<td>125 (59)†</td>
<td>97 (35)</td>
</tr>
</tbody>
</table>

*Data shown are means (SD) unless otherwise indicated. All variables were measured at baseline. Diabetes was defined as fasting glucose >7.0 mmol/L or current use of diabetic medications. IQR indicates interquartile range. †P<0.001; ‡P<0.05 compared with whites within gender. §To convert to SI units, multiply by 0.0259.

For the longitudinal analyses, the independent variables were the baseline procoagulants, and the outcome variables were follow-up IMT. The covariates were baseline age, race, gender, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. For the cross-sectional analyses, the independent variables were follow-up procoagulants, and the outcome variables were follow-up IMT. The covariates were follow-up age, race, gender, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. For the hemostatic group analyses, the covariates were baseline age, BMI, smoking, education, center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute Inc).

Results

Table 1 shows the baseline characteristics of the study participants according to gender–race group. Blacks were younger than whites, and black women had a greater BMI. Black participants had less education and more current smokers. Black women tended to have higher systolic blood pressures than white women, and black men had higher HDL cholesterol than white men. CRP levels were higher in blacks than whites. FVIII and vWF levels were higher in blacks than whites, and the mean values of all hemostatic factors were higher at follow-up (percentage increase: FVII, 48%; FVIII, 55%; and vWF, 40%).

Factor VII

Longitudinal associations between IMT and FVII were evaluated by examining baseline factor levels and IMT recorded at follow-up. After adjustment for age, race, and gender, there was a significant trend (P=0.007) of higher FVII levels with CC-IMT but not with IC-IMT (Figure 2A). On further analysis, the association of CC-IMT with FVII was restricted to whites (P<0.002) and men (P=0.015), but the trend was no longer apparent after multivariable adjustment for baseline age, BMI, smoking, education, field center, systolic blood pressure, diabetes, antihypertensive medication use, total and HDL cholesterol, and CRP level (data not shown). In regression analyses that included potential covariates one at a time into the age-, gender- and race-adjusted model, confounding was found to be primarily attributable to BMI.

Figure 2B displays a cross-sectional analysis of follow-up FVII levels and CC-IMT. Similar to the longitudinal data, significant trends were observed in the age, race-, and gender-adjusted model between FVII and CC-IMT in the total group (P=0.002), in whites (P=0.001), and men (P=0.007), but not with IC-IMT. Again, significance was lost on multivariable adjustment.

Next, we performed an analysis to examine whether having a FVII in the highest tertile at baseline, follow-up, or both would be associated with IMT. Significant associations were noted after adjustment for age, race, and gender between CC-IMT and participants having ≥1 FVII levels in the highest tertile in the total group and in whites (P<0.05) but not in blacks (Figure 3). Figure 3 also shows that significance was retained after imputation analysis for missing data. On multivariable analysis, significance was retained for whites (P<0.05) but not for the total group. No associations were observed for IC-IMT.

Factor VIII

With respect to FVIII, there was a significant association between baseline FVIII and IC-IMT for the total group (n=1083) after age, race, and gender adjustment (P=0.015) but not after multivariable adjustment. There was also a trend for an association between IC-IMT and whites (n=706; P=0.054) and women (n=586; P=0.034), but significance was not retained after multivariable adjustment. No significant associations were observed between follow-up FVIII and IC-IMT on cross-sectional analyses or after grouping participants according to whether FVIII was in the lowest or highest tertiles at baseline or follow-up.
Von Willebrand Factor

No significant associations were observed between vWF and IMT at either examination or after grouping participants according to whether vWF was in the lowest or highest tertiles at baseline or follow-up.

Discussion

A consistent finding of this study is the positive association of FVII with CC-IMT. This association was found whether FVII was measured when participants were 25 to 37 years of age or 38 to 50 years of age. Furthermore, it was observed in
participants having ≥1 FVII levels in the highest tertile at either age and was highly significant for whites and men. These associations between FVII and IMT were observed in age-/gender-/race-adjusted analyses but were attenuated after adjustment for BMI, total cholesterol, and HDL cholesterol, factors shown previously to be positively associated with FVII in young adults. Thus, FVII is not an independent risk factor for carotid thickness.

Nearly 30 years ago, the Northwick Park Heart Study described a strong association of FVII, FVIII, and fibrinogen with cardiovascular death in men 40 to 64 years of age at study inception. However, in a subsequent study, they found associations were weaker: hazard ratios for stroke were 1.36 (95% CI, 1.09 to 1.69) for fibrinogen and 1.07 (95% CI, 1.01 to 1.12) for FVII (Table 2). The Atherosclerosis Risk in Communities study measured hemostatic factors in nearly 15,000 participants 45 to 64 years of age initially free of CVD, followed up for at least 9 years.9 The adjusted relative risk for occurrence of ischemic stroke in those in the highest versus lowest fourth of clotting factor were 1.03 (95% CI, 0.88 to 1.21) for FVII, 1.93 (95% CI, 1.2 to 3.1) for FVIII, 1.71 (95% CI, 1.1 to 2.7) for vWF, and 1.26 (95% CI, 0.8 to 2.0) for fibrinogen. The Cardiovascular Health Study, an examination of nearly 6000 persons ≥65 years of age, found associations with stroke or transient ischemic attack in women for FVIII (relative risk, 1.4), and in men for fibrinogen (relative risk, 1.3).10 Although the initial examination of study data did not show a consistent association of FVII with stroke, on follow-up, a significant adjusted hazard ratio of 1.6 (95% CI, 1.2 to 2.2) was reported.11 They also observed that 6 single-nucleotide polymorphisms were associated with strokes, and 4 of these were in FVII-related genes.

Elevated levels of FVII have been reported in persons with carotid wall thickening and in men with coronary events. FVII is increased in women with coronary atheroma, and common FVII haplotypes contribute to the risk of myocardial infarction in women. However, the Glostrup study of 695 60-year-old Danish men and women did not find an association of FVII and carotid IMT. This suggests that the effect of FVII on atherogenesis is complex and influenced by a variety of risk factors. Our findings that the association of FVII with IMT is affected by BMI are in agreement with this view.

FVII is activated by tissue factor, and the FVIIa–tissue factor complex activates both intrinsic (FVIII, FIX) and extrinsic (FX) coagulation pathways. Raised FVII concentrations enhance thrombin generation and fibrin formation and contribute to vascular occlusion. In contrast to our observations regarding FVII, we found few associations of FVIII with carotid IMT, and none remained significant after multivariable adjustment. There were also no associations of vWF with IMT. Although increased FVIII has been associated with arterial vascular events before 50 years of age, such persons usually have a family history of elevated FVIII or vascular events. A possible explanation for why FVII, but not FVIII or vWF, is associated with subclinical atherosclerosis relates to the noncoagulant activity of FVII; it binds to tissue factor, and the complex induces smooth muscle cell proliferation.

A limitation of our study is that different methods were used to measure hemostatic factors at baseline and at follow-up. Activity assays were used at baseline, whereas antigen assays were used at follow-up. To determine the impact of this difference in assay methodology, we studied 40 healthy volunteers, matched for age and sex with the CARDIA participants. The correlation coefficients between activity and antigen assays were reasonably strong (0.81 for FVII, 0.61 for FVIII, and 0.95 for vWF). Thus, the increases in the hemostatic factors we observed during the 13-year interval were likely attributable to aging of the participants. Further, although the assay methods used in our study at baseline and follow-up were different, their relationships to other measurements, such as IMT, were very similar (Figure 2). Another limitation is that the changes in clotting factors we observed could be a consequence, rather than the cause of the carotid thickening.

In conclusion, the main finding of this study is that FVII levels in the highest tertile are associated with an increase in CC-IMT. This association was observed when FVII was measured at 25 to 37 years of age as well as at 38 to 50 years of age, but lost statistical significance after multivariable adjustment for other CVD risk factors, most prominently BMI. Our observations suggest that persistently high levels of FVII, beginning as early as 25 to 37 years of age, are associated with the appearance of carotid thickening 13 years later.

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### Disclosures
The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. There are no conflicts of interest to disclose.

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