



Universiteit
Leiden
The Netherlands

Decreased coagulability has no clinically relevant effect on atherogenesis: observations in individuals with a hereditary bleeding tendency

Rosendaal, F.R.

Citation

Rosendaal, F. R. (2001). Decreased coagulability has no clinically relevant effect on atherogenesis: observations in individuals with a hereditary bleeding tendency, *104*, 762-767. Retrieved from <https://hdl.handle.net/1887/1612>

Version: Not Applicable (or Unknown)

License:

Downloaded from: <https://hdl.handle.net/1887/1612>

Note: To cite this publication please use the final published version (if applicable).

Decreased Coagulability Has No Clinically Relevant Effect on Atherogenesis

Observations in Individuals With a Hereditary Bleeding Tendency

A Šrámek, MD, J H C Reiber, PhD, W B J Gerrits, MD, F R Rosendaal, MD

Background—Hemostasis affects ischemic cardiovascular disease through its role in formation of occluding arterial thrombi. Several studies suggest that hemostasis also might play a role in atherogenesis. We investigated whether individuals with an inherited bleeding tendency are protected against development of atherosclerosis.

Methods and Results—A total of 76 individuals with an inherited bleeding tendency (hemophilia and von Willebrand disease) and 142 healthy controls were included in the present study. Early atherosclerotic vessel-wall changes were quantified by measurement of intima-media thickness in the carotid and femoral arteries by B-mode ultrasonography. To validate intima-media thickness measurements, measurements also were performed in 77 individuals with clinically proven atherosclerosis and in 34 healthy, age-matched controls. A large difference in intima-media thickness was found between individuals with proven atherosclerosis and healthy controls, in particular for the femoral artery (difference for carotid artery, 0.16 mm, femoral artery, 0.53 mm). Comparison between patients with a bleeding tendency and healthy controls showed only minimally reduced intima-media in femoral artery in individuals with a bleeding tendency (adjusted difference, -0.078 mm, 95% CI, -0.17 to 0.018 mm). Subgroup analysis revealed that in subjects with moderate to severe hemophilia, vessel walls were thinnest (adjusted difference, -0.10 mm, 95% CI, -0.27 to 0.061 mm).

Conclusions—Hypocoagulability caused by hemophilia or von Willebrand disease has at most a limited effect on atherogenesis (*Circulation*. 2001;104:762-767.)

Key Words: atherosclerosis ■ coagulation ■ carotid arteries ■ femoral arteries ■ ultrasonics

Results of studies concerning the role of hemostasis in ischemic cardiovascular disease indicate that hypercoagulability increases risk of ischemic cardiovascular disease, whereas hypocoagulability decreases that risk. In many studies, high fibrinogen levels proved to be a risk factor for myocardial infarction and stroke.¹⁻⁶ Results for other clotting-factor levels, such as factor VII, are less consistent. In these studies, clotting-factor levels were in the normal or subnormal range. Patients with a hereditary deficiency of clotting factors VIII and IX (hemophilia A and B, respectively) have considerable protection against myocardial infarctions.^{7,8} Furthermore, increased risk of myocardial infarctions was found among carriers of a common prothrombin variant (20210 G to A) that is associated with increased prothrombin levels.^{9,10} Overall results of these studies indicate that coagulability plays a role in ischemic heart disease, particularly when extremes of coagulability are considered.

Myocardial infarction is the result of 2 processes: slow and chronic development of an atherosclerotic plaque and acute formation of an occluding thrombus at the site of the

plaque.^{11,12} Obviously, hemostasis plays an important role in formation of occluding arterial thrombi. However, results of a number of studies suggest that coagulability also might play a role in atherogenesis. Among patients who suffer from severe peripheral arterial disease, hereditary thrombophilia (caused by protein S deficiency) was more prevalent than in the general population.¹³ Furthermore, experimental animal studies showed that pigs with complete deficiency of von Willebrand factor were protected against spontaneous development of atherosclerotic plaques.^{14,15}

In the present study, we examined whether hypocoagulability protects against development of atherosclerotic plaques. Early atherosclerotic vessel wall changes were quantified by measuring intima-media thickness in superficial arteries by ultrasonography. Measurements were performed in patients with a hereditary bleeding tendency caused by a clotting-factor deficiency and in healthy controls for comparison. Individuals with a bleeding tendency were patients with hemophilia A or B or who suffered from mild von Willebrand disease. Because hemophiliacs are almost exclusively men,

Received February 2, 2001, revision received June 5, 2001, accepted June 7, 2001

From the Departments of Clinical Epidemiology (A Š, F R R), and Radiology (J H C R) and the Hemostasis and Thrombosis Research Center (F R R), Leiden University Medical Center, Leiden, and the Department of Hematology (W B J G), Leyenburg Hospital, The Hague, the Netherlands.

Correspondence to Prof Dr F R Rosendaal, Department of Clinical Epidemiology, CO-P46, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, Netherlands. E-mail: f.r.rosendaal@lumc.nl

© 2001 American Heart Association, Inc

Circulation is available at <http://www.circulationaha.org>

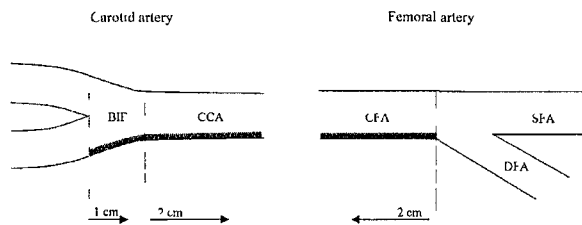


Figure 1. Schematic visual presentation of the measurement areas in the carotid (left) and femoral (right) arteries. Areas in the far wall at which measurements of intima-media thickness were performed are indicated by a thick gray line. BIF indicates carotid bifurcation, CCA, common carotid artery, CFA, common femoral artery, DFA, deep femoral artery, and SFA, superficial femoral artery.

the present study was restricted to men. Established risk factors of atherogenesis were determined to adjust for differences among groups.

Methods

Study Design

To validate the methodology, measurements were performed in 77 men who had undergone coronary bypass surgery and in 34 healthy, age-matched men. To determine the role of hypocoagulability in atherogenesis, 76 men with congenital bleeding tendency caused by a deficiency of clotting factors VIII or IX or von Willebrand factor were included. All male patients ≥ 30 years of age who were treated in the 2 participating centers (Leiden and Den Haag) were eligible and asked to participate. The group consisted of 17 patients with heterozygous von Willebrand disease (mean factor VIII antigen, 45 IU/dL, range, 18 to 97 IU/dL, mean von Willebrand factor antigen, 36 IU/dL, range, 12 to 65 IU/dL) and 34 with mild (>5 IU/dL), 5 with moderate (1 to 5 IU/dL), and 20 with severe (<1 IU/dL) hemophilia (52 with hemophilia A and 7 with hemophilia B). For comparison, 108 individuals were included who had received temporarily prophylactic anticoagulant treatment because of an orthopedic condition such as surgery or fractures. The 34 healthy individuals from the validation study were added, which resulted in a total of 142 healthy male controls. At the time of study, none of the controls was using anticoagulant therapy. The present study was approved by the Medical Ethics Committee of Leiden University Medical Center, and all subjects gave informed consent.

Ultrasound

We used an Aloka SSD 1200 ultrasound machine with a 7.5-MHz linear transducer. An elaborate description of the technique and image analysis is given elsewhere.¹⁶ In brief, 2 frozen images of the intima-media complex in the common carotid artery of both sides (4 images total) were saved on an S-VHS videotape. Images were frozen during the R phase of the heart cycle by use of an ECG triggering module attached to the ultrasound machine. Similarly, 4 images in the carotid bifurcation and common femoral artery were recorded. At a later stage, images were digitized in a personal computer and saved on a recordable CD. All images for each individual were saved under a single, randomly allocated identification number to ensure matching of the subject's identity during later measurements. Images were magnified 4 times to visualize clearly the intima-media complex in the far wall as has been defined by Pignoli et al.¹⁷ In every image, intima-media thickness of the far wall was measured 6 times at constant intervals and averaged by use of dedicated software.¹⁸ Figure 1 is a schematic that shows areas in the carotid and femoral arteries at which measurements were performed. Mean intima-media thickness in carotid artery was calculated by averaging measurements of images of the common carotid artery and carotid bifurcation. Mean thickness in the femoral artery was based on

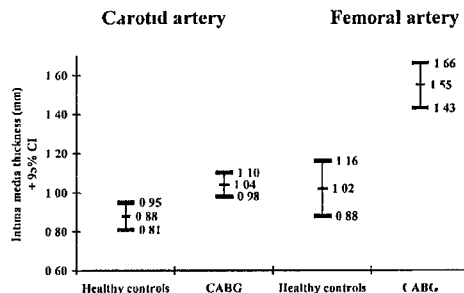


Figure 2. Visual comparison of mean intima-media thickness in carotid and femoral arteries between coronary bypass patients and healthy, age-matched controls. Mean and 95% confidence intervals of intima-media thickness (in mm) are indicated by —.

measurements from images of the common femoral artery. Image acquisition and measurements were performed by 1 individual (AŠ).

Measurement of Risk Factors

Information about use of medication, smoking habits, and presence of cardiovascular disease (myocardial infarction and stroke) before the age of 65 years for first-degree relatives was obtained by use of a standardized questionnaire. Smoking was categorized as number of pack-years an individual had smoked. Body-fat distribution was determined by measurement of waist-to-hip ratio. For each subject, blood pressure was measured 3 times by use of a Hawksley random-zero mercury sphygmomanometer while the subject was in a supine position and after ≥ 20 minutes of rest. From each individual, we drew fasting blood samples (serum and plasma). Fractions of VLDL, LDL, and HDL determinations were obtained by ultracentrifugation of serum samples. Total serum cholesterol, HDL, LDL, VLDL, and triglyceride concentrations were determined by use of an enzymatic colorimetric method.

Statistical Analysis

Vessel-wall thickness of patients and controls was compared by Student's *t* test for continuous variables and by χ^2 test for discrete variables, after log-transformation when appropriate. Association of all determinants studied with intima-media thickness was quantified by linear regression. For univariate regression analysis, including blood pressure, we excluded 27 individuals who used antihypertensive drugs, 7 individuals from the regression analysis of lipids were excluded because they used lipid-lowering drugs. Multivariate regression analysis was used to correct for differences in exposure to established risk factors.

Results

Patients with coronary bypasses had substantially thicker vessel walls both in the carotid and femoral arteries than healthy, age-matched controls (Figure 2). For the carotid artery, mean difference was 0.16 mm, for the femoral artery, 0.53 mm.

Table 1 shows general characteristics and presence of various risk factors for patients with a bleeding tendency and healthy controls. Mean age of patients was 49 years (range, 31 to 84) and was similar to mean age of controls (mean, 52 years, range, 30 to 76). Exposure to risk factors for atherosclerosis was similar. Percentage of persons with a bleeding tendency who used antihypertensive or cholesterol-lowering drugs (16% and 5%, respectively) was slightly higher than in controls (11% and 2%, respectively). Frequency of hypertension (either drug-treated or high blood pressure) was similar for patients and controls (18% versus 17%, respectively).

TABLE 1. General Characteristics of Patients With a Clotting Disorder and Healthy Individuals

| Variables | Bleeding Tendency (n=76) | Healthy Controls (n=142) | 95% CI of Difference |
|-------------------------------|-----------------------------|-----------------------------|-------------------------|
| Mean age, y | 48.8 | 51.5 | -6.2-0.7 |
| Smoking, pack y | 16.12 | 13.47 | -1.5-3.2* |
| Waist-to-hip ratio | 0.97 | 0.97 | -0.017-0.015 |
| Blood pressure, mm Hg | | | |
| Systolic | 117.9 | 119.0 | -3.0-1.0* |
| Diastolic | 76.6 | 77.0 | -3.37-2.61 |
| Total cholesterol, mmol/L | 5.2 | 5.4 | -0.4-0.2 |
| VLDL, mmol/L | 0.90 | 0.89 | -1.12-3.08* |
| LDL, mmol/L | 3.3 | 3.3 | -0.2-0.2 |
| HDL, mmol/L | 0.94 | 0.98 | -0.12-0.045 |
| Triglycerides, mmol/L | 1.2 | 1.1 | -0.20-0.25* |
| Positive family history, % | 19 (25) | 30 (21) | -8-16 |
| Cholesterol-lowering drugs, % | 4 (5) | 3 (2) | -2-8 |
| Antihypertension drugs, % | 12 (16) | 15 (11) | -4-15 |
| Hypertension, % | 14 (18) | 24 (17) | -9-12 |
| Diabetes, % | 3 (4) | 2 (1) | -2-7 |

Hypertension was defined as systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertension drugs

*After logarithmic transformation

For the intima-media thickness in the carotid artery, we observed no difference between healthy controls and patients with a bleeding tendency (Figure 3). Figure 4 shows results for the femoral artery. Mean intima-media thickness was slightly lower for patients with a bleeding tendency, this effect appeared restricted to those with hemophilia (mean difference after logarithmic transformation, 0.11 mm, 95% CI, -0.0028 to 0.22).

In univariate linear regression analysis, most of the established risk factors for atherosclerosis showed a clear relation to intima-media thickness in both arteries (Table 2). Except for HDL, variables showed a more-pronounced effect on thickness in the femoral artery than in the carotid artery. This applied particularly for smoking (coefficient, 0.022 versus 0.068), total cholesterol (coefficient, 0.035 versus 0.11), LDL (coefficient, 0.035 versus 0.14), and presence of hypercho-

lesterolemia (coefficient, 0.070 versus 0.23). Univariate regression analysis of intima-media thickness between controls and individuals with a clotting disorder showed results similar to those shown in Figures 3 and 4: no difference in intima-media thickness of the carotid artery but a slightly thinner femoral intima-media in individuals with a bleeding tendency (-0.091 mm, 95% CI, -0.20 to 0.022 mm), particularly in those with hemophilia (-0.11, 95% CI, -0.23 to 0.016 mm).

Adjustment for established risk factors attenuated differences between controls and patients, but intima-media remained thinner among patients with a coagulation disorder compared with controls (Table 3). This occurrence was true especially in the comparison between patients suffering from moderate to severe hemophilia and controls (adjusted difference, -0.10 mm, 95% CI, -0.27 to 0.061).

Carotid artery

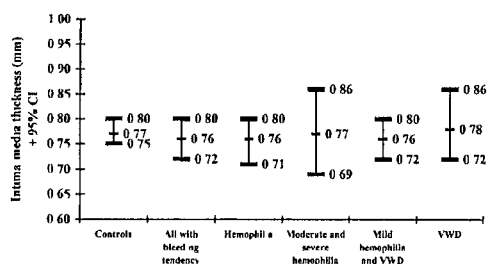


Figure 3. Visual comparison of mean intima-media thickness in carotid artery between healthy individuals and subgroups of individuals with a bleeding tendency. Mean and 95% confidence intervals of the intima-media thickness (in mm) are indicated by —. VWD indicates von Willebrand disease.

Discussion

In the present study, we examined whether hypocoagulability results in less atherosclerotic vessel wall changes than normal coagulation. As a model for hypocoagulability, we selected patients suffering from a hereditary deficiency of clotting factor VIII or IX or von Willebrand factor. We observed no clinically relevant effect of decreased coagulability on atherogenesis.

We used B-mode ultrasonography to quantify early atherosclerotic vessel wall changes. In a recent study, we showed that the ultrasound technique used in the present study is accurate and reliable for determination of intima-media thickness in the carotid and femoral arteries.¹⁶ Numerous studies found a clear relation between intima-media thickness in the carotid artery and most of the established risk factors, even in

TABLE 2. Univariate Regression Analysis of Intima-Media Thickness in Carotid and Femoral Arteries

| Variables | Artery | | | |
|-------------------------------|-------------|--------------|-------------|-------------|
| | Carotid | | Femoral | |
| | Coefficient | 95% CI | Coefficient | 95% CI |
| Age (10 y) | 0.10 | 0.087–0.12 | 0.16 | 0.12–0.19 |
| Smoking (10 pack-y) | 0.022 | 0.0071–0.039 | 0.068 | 0.035–0.10 |
| Waist-to-hip ratio | 1.05 | 0.63–1.48 | 1.60 | 0.67–2.51 |
| Blood pressure (10 mm Hg) | | | | |
| Systolic | 0.046 | 0.033–0.060 | 0.085 | 0.055–0.11 |
| Diastolic | 0.038 | 0.014–0.061 | 0.092 | 0.042–0.14 |
| Total cholesterol (mmol/L) | 0.035 | 0.011–0.059 | 0.11 | 0.067–0.16 |
| VLDL (mmol/L) | 0.065 | 0.015–0.12 | 0.13 | 0.026–0.23 |
| LDL (mmol/L) | 0.035 | 0.0039–0.065 | 0.14 | 0.075–0.20 |
| HDL (mmol/L) | –0.057 | –0.15–0.033 | 0.018 | –0.17–0.20 |
| Triglycerides (mmol/L) | 0.036 | 0.0080–0.059 | 0.070 | 0.018–0.12 |
| Positive family history | 0.054 | –0.0064–0.12 | 0.11 | –0.016–0.24 |
| Hypertension | 0.20 | 0.13–0.26 | 0.28 | 0.14–0.42 |
| Hypercholesterolemia | 0.070 | 0.015–0.13 | 0.23 | 0.12–0.35 |
| All with bleeding tendency | –0.0093 | –0.063–0.044 | –0.091 | –0.20–0.022 |
| Hemophilia | –0.013 | –0.073–0.046 | –0.11 | –0.23–0.016 |
| Moderate to severe hemophilia | 0.0060 | –0.078–0.090 | –0.097 | –0.28–0.084 |
| Mild hemophilia and VWD | –0.017 | –0.077–0.043 | –0.088 | –0.22–0.043 |
| VWD | 0.0043 | –0.089–0.098 | –0.033 | –0.25–0.18 |

VWD indicates von Willebrand disease. Hypertension was defined as systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertension drugs. Hypercholesterolemia was defined as total cholesterol > 6 or use of cholesterol-lowering drugs. Regression coefficient determined for subgroups with a bleeding tendency indicates difference in intima-media thickness (mm) between subgroups and controls (ie intima-media thickness of subgroup minus intima-media thickness of controls).

young individuals^{19–22} Intima-media thickness in the carotid and femoral arteries is a good indicator of presence of atherosclerosis in coronary and other peripheral arteries.^{23,24} We also found a clear effect of established risk factors on intima-media thickness. Furthermore, we found a large difference in intima-media thickness between patients with clinically proven coronary atherosclerosis and healthy controls.

Numerous studies have been performed about the role of coagulation and clotting factor levels in ischemic cardiovas-

cular disease. In particular, fibrinogen emerged as a risk factor from these studies.^{1,6,25} However, in these studies, clinical end points of cardiovascular disease such as myocardial infarction or stroke were used. Therefore, these studies do not clarify whether coagulation plays a role only in arterial thrombosis or also in atherogenesis. Several studies used carotid intima-media thickness measurements as a surrogate end point of ischemic cardiovascular disease. Results of these studies show no consistent results with respect to association of clotting factor levels with early atherosclerotic changes.^{19,26–28} A possible explanation for this inconsistency is that clotting-factor levels in individuals studied were mainly in the normal to subnormal range. In the present study, we maximized contrast by including patients with hereditary low clotting-factor levels. Because patients were selected on the basis of a genetic trait with high penetrance, selection and confounding are unlikely.

Our results suggest that highly decreased clotting-factor levels may have a small protective effect against atherosclerosis in the femoral artery and no effect in the carotid artery. Previous studies have suggested that different risk factor patterns exist for the carotid and femoral arteries.^{29,30} This variance might be caused by different hemodynamic conditions within these arteries, such as differences in shear stress of the wall and other factors.³¹ Another, perhaps more

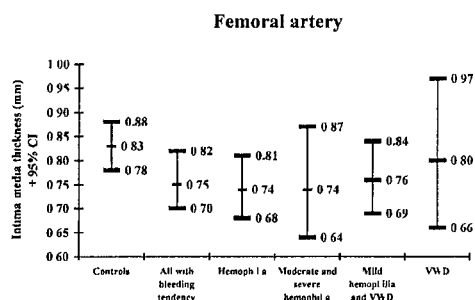


Figure 4. Visual comparison of mean intima-media thickness in femoral artery between healthy individuals and subgroups of individuals with a bleeding tendency. Mean and 95% confidence intervals of the intima-media thickness (in mm) are indicated by —

TABLE 3. Comparison of Intima-Media Thickness in Carotid and Femoral Arteries of Subgroups of Patients With a Bleeding Disorder to Group of Controls After Multivariate Regression Analysis

| Subgroup | Artery | | | |
|-------------------------------|-------------|--------------|-------------|-------------|
| | Carotid | | Femoral | |
| | Coefficient | 95% CI | Coefficient | 95% CI |
| All with bleeding tendency | 0.0091 | -0.030-0.048 | -0.078 | -0.17-0.018 |
| Hemophilia | 0.0047 | -0.040-0.049 | -0.087 | -0.20-0.020 |
| Moderate to severe hemophilia | -0.016 | -0.081-0.048 | -0.10 | -0.27-0.061 |
| Mild hemophilia and VWD | -0.017 | -0.077-0.043 | -0.088 | -0.22-0.043 |
| VWD | 0.028 | -0.044-0.099 | -0.068 | -0.25-0.11 |

VWD indicates von Willebrand disease. Variables adjusted for in the regression analysis were age, smoking habits, waist-to-hip ratio, systolic blood pressure, total cholesterol, VLDL, LDL, HDL, triglycerides, family history of cardiovascular disease, use of cholesterol-lowering drugs, and use of antihypertension drugs. Regression coefficient determined for subgroups with a bleeding tendency indicates adjusted difference in intima-media thickness (mm) between subgroup and controls.

plausible, explanation is that measurements in the femoral artery are more sensitive than those in the carotid artery for detection of early atherosclerotic changes. In the comparison between patients with proven coronary atherosclerosis and healthy controls, we found a larger difference in intima-media thickness in the femoral artery than in the carotid artery. This finding suggests that minor changes are detected better in the femoral than carotid artery.

Our results indicate minor effects on atherosclerosis in individuals with extreme hypocoagulability. In a recently published study, presence of advanced atherosclerotic lesions in the carotid artery between patients suffering from hemophilia A and von Willebrand disease were compared with healthy controls.³² Results suggested considerable protection in the patients with a clotting-factor deficiency, even in those suffering from mild von Willebrand disease. However, in that study, the selection procedure and a general characteristic of the study groups (ie, age) were not documented. Furthermore, only crude data were presented and no adjustments were performed. In experimental animal studies with pigs, protection against atherosclerosis was found in pigs with severe von Willebrand disease, whereas no protection was found in pigs suffering from the mild type of the disease.^{14,33} These results are not in disagreement with our results, because we included only subjects with mild von Willebrand disease (albeit in only a small number of patients).

We studied hereditary deficiencies of clotting factor VIII and IX and von Willebrand factor as a model for hypocoagulability of blood. Deficiencies of these clotting factor levels will result in less formation of thrombin and fibrin (impaired secondary hemostasis) or less platelet aggregation (impaired primary hemostasis). The present model is valid only for the hypotheses in which incorporation of mural thrombi in the vessel wall, mitogen, and other atherosclerotic effects of an increased thrombin and fibrin production are a part of the pathway to atherosclerosis. Some studies suggest that fibrinogen also might have an effect on atherogenesis through its effect on the viscosity of blood. In recent studies, viscosity of blood emerged as an important risk factor for atherosclerotic disease.^{34,35}

In the present study, patients with a bleeding tendency were treated temporarily to correct the clotting disorder during hemorrhages. Some patients received prophylactic treatment to avoid hemorrhages. During periods of prophylaxis and treatment of hemorrhages, the coagulation levels of these patients were increased. A study concerning treatment of hemophilia in the Netherlands³⁶ showed that ≈50% of patients who suffered from severe hemophilia (in particular, younger individuals) receive prophylactic treatment. Prophylactic dosage level is determined so that mean clotting-factor VIII or IX level will be increased by 0.05 to 0.1 IU/mL. Thus, during prophylactic treatment, severity of hemophilia is "converted" from severe to mild. Patients with mild hemophilia usually receive no prophylactic treatment. During bleedings, treatment is aimed at a higher increase in clotting factors. However, treatment in these cases is given only for a period of several days. The same study showed that patients with moderate or severe hemophilia experience a bleeding episode every 3 to 4 weeks, whereas those with mild hemophilia rarely bleed spontaneously. Therefore, treatment has only a limited effect on overall coagulability. Even in case of prophylactic treatment, a high contrast remains in lifetime coagulability between individuals with a severe bleeding disorder and individuals with normal coagulation.

In conclusion, results of the present study suggest a minimal protective effect of extreme hypocoagulability on atherogenesis. Our results indicate that the protection against myocardial infarctions that has been reported in hemophilia patients probably is primarily caused by a decreased tendency to form occluding arterial thrombi. Because we compared early atherosclerotic changes between individuals with a high contrast in coagulability, the protective effect is negligible in individuals with coagulation in the normal or subnormal range.

Acknowledgments

The present study was funded by the Netherlands Heart Foundation (No. 93.110). We express our gratitude to all individuals who participated in this study. We thank M. Horstmannshoff for secretarial and administrative support.

References

- 1 Wilhelmssen L, Svardsudd K, Korsan-Bengtson K, et al Fibrinogen as a risk factor for stroke and myocardial infarction *N Engl J Med* 1984,311 501–505
- 2 Meade TW, Mellows S, Brozovic M, et al Haemostatic function and ischaemic heart disease principal results of the Northwick Park Heart Study *Lancet* 1986,2 533–537
- 3 Cortellaro M, Boschetti C, Cofrancesco E, et al The PLAT Study hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients principal results PLAT Study Group Progetto Lombardo Atero-Trombosi (PLAT) Study Group *Arterioscler Thromb* 1992,12 1063–1070
- 4 Smith FB, Lee AJ, Fowkes FG, et al Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study *Arterioscler Thromb Vasc Biol* 1997,17 3321–3325
- 5 Folsom AR, Rosamond WD, Shahar E, et al Prospective study of markers of hemostatic function with risk of ischemic stroke The Atherosclerosis Risk in Communities (ARIC) Study Investigators *Circulation* 1999,100 736–742
- 6 Doggen CJ, Manger C, V, Bertina RM, et al A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men *Thromb Haemost* 1998,80 281–285
- 7 Rosendaal FR, Briet E, Stubbe J, et al Haemophilia protects against ischaemic heart disease a study of risk factors *Br J Haematol* 1990,75 525–530
- 8 Triemstra M, Rosendaal FR, Smit C, et al Mortality in patients with hemophilia changes in a Dutch population from 1986 to 1992 and 1973 to 1986 *Ann Intern Med* 1995,123 823–827
- 9 Rosendaal FR, Siscovick DS, Schwartz SM, et al A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women *Blood* 1997,90 1747–1750
- 10 Doggen CJ, Cats VM, Bertina RM, et al Interaction of coagulation defects and cardiovascular risk factors increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A *Circulation* 1998,97(11) 1037–1041
- 11 Davies MJ, Thomas A Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death *N Engl J Med* 1984,310 1137–1140
- 12 Falk E, Shah PK, Fuster V Coronary plaque disruption *Circulation* 1995,92 657–671
- 13 Allaart CF, Aronson DC, Ruys T, et al Hereditary protein S deficiency in young adults with arterial occlusive disease *Thromb Haemost* 1990,64 206–210
- 14 Fuster V, Lie JT, Badimon L, et al Spontaneous and diet-induced coronary atherosclerosis in normal swine and swine with von Willebrand disease *Arteriosclerosis* 1985,5 67–73
- 15 Fuster V, Fass DN, Kaye MP, et al Arteriosclerosis in normal and von Willebrand pigs long-term prospective study and aortic transplantation study *Circ Res* 1982,51 587–593
- 16 Šrámek A, Bosch JG, Reiber JH, et al Ultrasound assessment of atherosclerotic vessel wall changes reproducibility in carotid and femoral arteries *Invest Radiol* 2000,35 699–707
- 17 Pignoli P, Tremoli E, Poli A, et al Intimal plus medial thickness of the arterial wall a direct measurement with ultrasound imaging *Circulation* 1986,74 1399–1406
- 18 Koning G, Reiber JH, von Land CD, et al Advantages and limitations of two software calipers in quantitative coronary arteriography *Int J Card Imaging* 1991,7 15–30
- 19 Sosef MN, Bosch JG, van Oostayen J, et al Relation of plasma coagulation factor VII and fibrinogen to carotid artery intima-media thickness *Thromb Haemost* 1994,72 250–254
- 20 Salonen R, Salonen JT Determinants of carotid intima-media thickness a population-based ultrasonography study in eastern Finnish men *J Intern Med* 1991,229 225–231
- 21 Heiss G, Sharrett AR, Barnes R, et al Carotid atherosclerosis measured by B-mode ultrasound in populations associations with cardiovascular risk factors in the ARIC study *Am J Epidemiol* 1991,134 250–256
- 22 Paucullo P, Iannuzzi A, Sartorio R, et al Increased intima-media thickness of the common carotid artery in hypercholesterolemic children *Arterioscler Thromb* 1994,14 1075–1079
- 23 Bots ML, Hofman A, Grobbee DE Common carotid intima-media thickness and lower extremity arterial atherosclerosis The Rotterdam Study *Arterioscler Thromb* 1994,14 1885–1891
- 24 Burke GL, Evans GW, Riley WA, et al Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults The Atherosclerosis Risk in Communities (ARIC) Study *Stroke* 1995, 26 386–391
- 25 Jansson JH, Nilsson TK, Johnson O, von Willebrand factor in plasma a novel risk factor for recurrent myocardial infarction and death *Br Heart J* 1991,66 351–355
- 26 Folsom AR, Wu KK, Shahar E, et al Association of hemostatic variables with prevalent cardiovascular disease and asymptomatic carotid artery atherosclerosis the Atherosclerosis Risk in Communities (ARIC) Study Investigators *Arterioscler Thromb* 1993,13 1829–1836
- 27 Agewall S, Wikstrand J, Suurkula M, et al Carotid artery wall morphology, haemostatic factors and cardiovascular disease an ultrasound study in men at high and low risk for atherosclerotic disease *Blood Coagul Fibrinolysis* 1994,5 895–904
- 28 Tracy RP, Bovill EG, Yanez D, et al Fibrinogen and factor VIII, but not factor VII, are associated with measures of subclinical cardiovascular disease in the elderly results from the Cardiovascular Health Study *Arterioscler Thromb Vasc Biol* 1995,15 1269–1279
- 29 Giral P, Pithois-Merli I, Filhitt V, et al Risk factors and early extracoronary atherosclerotic plaques detected by three-site ultrasound imaging in hypercholesterolemic men Prevention Cardio-vasculaire en Medecine du Travail METRA Group *Arch Intern Med* 1991,151 950–956
- 30 Joensuu T, Salonen R, Winblad I, et al Determinants of femoral and carotid artery atherosclerosis *J Intern Med* 1994,236 79–84
- 31 Glagov S, Zarins C, Giddens DP, et al Hemodynamics and atherosclerosis insights and perspectives gained from studies of human arteries *Arch Pathol Lab Med* 1988,112 1018–1031
- 32 Bilora F, Dei RC, Girolami B, et al Do hemophilia A and von Willebrand disease protect against carotid atherosclerosis? A comparative study between coagulopathics and normal subjects by means of carotid echo-color Doppler scan *Clin Appl Thromb Hemost* 1999,5 232–235
- 33 Badimon L, Steele P, Badimon JJ, et al Aortic atherosclerosis in pigs with heterozygous von Willebrand disease comparison with homozygous von Willebrand and normal pigs *Arteriosclerosis* 1985,5 366–370
- 34 Lee AJ, Mowbray PI, Lowe GD, et al Blood viscosity and elevated carotid intima-media thickness in men and women The Edinburgh Artery Study *Circulation* 1998,97 1467–1473
- 35 Lowe GD, Lee AJ, Rumley A, et al Blood viscosity and risk of cardiovascular events the Edinburgh Artery Study *Br J Haematol* 1997,96 168–173
- 36 Triemstra M, Smit C, Van der Ploeg HM, et al Two decades of haemophilia treatment in the Netherlands *Haemophilia* 1995,1 165–171