 Associations between cardiovascular risk factors, hyper- and hypocoagulability
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Chapter 10

Factor VIII deficiency does not protect against atherosclerosis

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Submitted
ABSTRACT

**Background** Hemophilia A patients have a lower cardiovascular mortality compared to the general population. Whether this protection is caused by hypocoagulability or decreased atherogenesis is unclear. We evaluated atherosclerosis and endothelial function in hemophilia A patients with and without obesity as well as in matched unaffected controls.

**Methods and Results** Fifty-one obese (body mass index (BMI) $\geq 30$ kg/m$^2$) and 47 non-obese (BMI $\leq 25$ kg/m$^2$) hemophilia A patients, and 42 obese and 50 matched non-obese male controls were included. Carotid and femoral intima media thickness (CIMT and FIMT) and brachial flow mediated dilatation (FMD) were measured, as markers of atherogenesis and endothelial function. Overall, population age was 50 ±13 years. CIMT was increased in obese (0.77 ± 0.22 mm) compared to non-obese subjects (0.69 ± 0.16 mm), (mean $\Delta$ 0.07 mm (95%CI 0.02-0.13 mm, p=0.008). No differences in mean CIMT and FIMT between obese hemophilia patients and obese controls were found (mean $\Delta$ 0.02 mm (95% CI -0.07-0.11, p=0.67) and 0.06 mm (95% CI -0.13-0.25, p=0.55), respectively). Thirty-five percent of the obese hemophilia patients and 29% of the obese controls had an atherosclerotic plaque (p=0.49), irrespective of the severity of hemophilia. FMD was comparable between obese hemophiliacs and obese controls (4.84% ± 3.24 and 5.32% ± 2.37, p=0.45).

**Conclusion** Hemophilia A patients with and without obesity have the same degree of atherosclerosis and endothelial function as their matched controls. These findings suggest that the lower cardiovascular mortality in hemophilia patients is likely caused by a decreased risk of arterial thrombosis.
BACKGROUND

Studies assessing the role of hemostasis in ischemic cardiovascular disease (CVD) indicate that hypercoagulability increases the risk of CVD, whereas a bleeding tendency seems to be associated with a lower risk. Previous studies showed that high levels of factor VIII are associated with an increased risk of both venous and arterial thrombosis, and numerous other coagulation factors have also been related to an increased thrombotic risk. The opposite also holds true, since patients with a hereditary deficiency of factor VIII (hemophilia A) experience a considerable protection against mortality due to CVD. In a recent meta-analysis it was shown that compared to the general population, patients with hemophilia had a 50% reduction in mortality due to ischemic heart disease.

Two processes are required for an arterial thrombotic event to occur: atherogenesis, which gradually leads to the development of an atherosclerotic plaque, and atherothrombosis, the acute formation of an occluding thrombus. A role of hemostasis in the formation of an occluding thrombus is evident, but coagulation factors such as factor VIII may also be involved in atherogenesis.

Whether hemophilia A protects against atherogenesis is unclear. Although factor VIII deficiency seemed to protect against atherogenesis in some animal and human studies, other studies found no association. However, the relative low incidence of cardiovascular events in this group of patients requires large population based studies. A major drawback of previous studies in hemophilia patients is the relative absence of cardiovascular risk factors. Measurement of the carotid and femoral intima media thickness (IMT) and endothelial function by means of brachial flow mediated dilatation (FMD) allow early detection of atherosclerosis or functional vessel wall abnormalities, but comparing subjects with a low prevalence of risk factors may not be the best model to distinguish differences in subclinical atherosclerosis. Studies assessing the prevalence of atherosclerosis in hemophilia patients with pro-atherosclerotic risk factors are lacking. Obesity is such an established and major cardiovascular risk factor. In addition, obesity is equally prevalent in the hemophilia population as in the general population.

To test our hypothesis that a lifelong hypocoagulable state (i.e. factor VIII deficiency) lowers the formation of atherosclerosis, we investigated the relationship between hemophilia A and the extent of atherosclerosis in a multicenter cross-sectional study. To assess subclinical atherosclerosis and (impaired) vascular function we measured IMT and FMD in hemophilia A patients with and without obesity and controls who were matched for age, gender and body mass index (BMI).
METHODS

Participating centers
Hemophilia A patients were recruited from various hemophilia treatment centers across the Netherlands and Belgium. Enrollment in the study took place in three study centers: the Academic Medical Center in Amsterdam, the Netherlands (AMC), University Medical Center Utrecht in Utrecht (UMCU), the Netherlands, and the University Hospital in Leuven (UZL), Belgium. The study was approved by the local ethics committees and inclusion took place after informed consent had been obtained.

Study population
Hemophilia A patients older than 18 years who had a BMI $\geq 30$ kg/m$^2$ were eligible for inclusion, irrespective of the severity of hemophilia. These patients were matched for age and severity of disease with non-obese hemophilia A patients ($\text{BMI} \leq 25$ kg/m$^2$). Hemophilia A patients with a history of symptomatic atherosclerotic disease (i.e. ischemic heart disease, stroke or peripheral vascular disease) or HIV were excluded.

Controls were matched to hemophilia patients for BMI, age and gender. These controls were recruited through placement of an advertisement in local newspapers or were healthy volunteers who had participated in other studies on cardiovascular disease at the Department of Vascular Medicine of the AMC, The Netherlands. To improve accrual, an obesity clinic in the Netherlands was also approached to identify suitable control subjects.

Study regimen and assessment of risk factors
All study subjects were invited to one of the centers after an overnight fast. Patients were instructed to refrain from food and drinks, except water, in the 10 hours prior to each measurement. Furthermore, to avoid any influence of a vena puncture on the FMD measurements, patients were asked to refrain from prophylactic infusion of factor VIII prior to the visit. Patients with a severe form of hemophilia however received factor VIII prophylaxis after blood collection, to avoid any bleeding complications as a result of the prolonged blood pressure cuff inflation during the FMD measurement. The study visit included measurement of the carotid and femoral IMT and brachial FMD by means of ultrasonography; a vena puncture to assess fasting glucose and lipid levels; and a physical examination including measurement of weight, length, waist and hip circumference. In all subjects, the ultrasound measurements preceded blood collection. Blood pressure was measured three times in a supine position during the IMT assessment and the last measurement was registered. Additionally, a medical history was obtained. Total cholesterol (TC) levels, LDL cholesterol and triglyceride levels were considered to be increased when they exceeded the 95th percentile of the reference values for the relevant age categories. HDL levels were considered to be low when they were below the fifth percentile of the reference values for the relevant age categories. Dyslipidemia was defined as the use of lipid lowering drugs and/or any of the cholesterol levels
exceeding the reference values for the relevant age categories. Diabetes mellitus was defined as the use of anti-diabetes medication and/or fasting glucose levels higher than 7.1 mmol/L. Hypertension was defined as the use of antihypertensive medication, a systolic blood pressure >140 mmHg, and/or a diastolic blood pressure of > 90 mmHg.

**Ultrasonography**

*Assessment of carotid and femoral intima-media thickness (IMT)*

For assessment of carotid and femoral ultrasound intima-media thickness (IMT) measurements, Acuson Sequoia instruments (Siemens Medical Solutions, Erlangen, Germany) equipped with linear-array ultrasound transducers (L7, 5–12 MHz) were used in all three study centers. Sonographers were trained and certified. Instrument application and scanning protocols were standardized as described in extenso previously. In each subject three arterial wall segments of the right and three segments of the left carotid artery were scanned; in each of the femoral arteries, one segment was scanned. In each center a maximum of two sonographers performed the ultrasound procedures. High-resolution images of each of the segments were saved as DICOM (Digital Imaging and Communication) in the diastole of the vessel. From all the centers, scans were transmitted by secure file transfer protocol (sFTP) to the AMC Vascular Imaging core laboratory. Image analyses was done by one certified ultrasound analyst (reader). For carotid and femoral IMT analyses eTRACK software (Vascular Imaging and Department of Physiology, AMC, Amsterdam, the Netherlands) was used. The reader was blinded to demographic and clinical information of subjects.

The primary ultrasound outcome was the per subject mean IMT of the six carotid arterial wall segments (mean carotid IMT). All other IMT outcomes, such as the per subject average maximum IMT of the six carotid segments (max carotid IMT), used to assess the presence of plaque, as well as the femoral IMT were secondary endpoints. To assess intrasonographer reproducibility and for quality control (QC) purposes repeat scans were assessed in 17 subjects. The observed mean difference of mean CIMT was 0.077 mm, which was far within the predefined intrasonographer QC limits of 0.2 mm.

**Plaques**

Since there is no clear consensus on the definition of plaques in the femoral artery, we only analysed the presence of atherosclerotic plaque in the carotid artery. All segments of the carotid artery were assessed for the presence of atherosclerotic plaques. A plaque was predefined as a maximum IMT ≥ 1.3 mm of any given segment of the carotid artery.

*Assessment of endothelial function by means of brachial flow mediated dilatation (FMD)*

Instrument application and scanning protocols were standardized as described previously. Sonographers were trained and certified. Each study subject underwent measurement of endothelium-dependent vascular responses of the left brachial artery by B-mode ultrasound imaging. Acuson Aspen (Siemens, Mountain View USA) ultrasound systems equipped with L7,
5-10 MHz linear arrays were used. Prior to starting the FMD scan subjects rested for at least 10 minutes in a quiet and temperature-controlled (21°C to 23°C) examination room. In all subjects the left brachial artery was scanned. Subsequently, the subjects’ left arm was placed in a custom made transducer holder with arm support and a blood pressure cuff was placed on the left forearm from the medial epicondyle downwards. A straight, non-branching segment of the brachial artery above the antecubital fossa was identified and scanned longitudinally. Following optimisation of depth and gain settings, end-diastolic brachial artery diameters were recorded at a beat-to-beat interval for 1 minute (baseline measurement). The cuff was then inflated to 250 mm Hg on the forearm for 5 minutes after which the cuff was deflated and the segment of the brachial artery was recorded continuously for another 3 minutes. Clips were stored on magnetic optical disc. Brachial artery diameter was analysed qualitatively and quantitatively offline by certified image analyst at the core lab of AMC Vascular imaging, Amsterdam. For image assessments, a validated automatic edge-detection system (Brachial Analyser, Medical Imaging Applications LLC, USA) was used. FMD was expressed as the % of the difference between the maximum post-cuff release brachial diameter and average pre-cuff inflation (‘baseline’) diameter. For brachial FMD QC purposes 19 repeat scans were available. With a pre-set intrasonographer QC limit of a mean difference FMD smaller than 2%, the sonographers and image analysts met the predefined criteria by far: the mean difference of paired repeat brachial FMD scans was 0.79%.

**Statistical analysis**

Data are presented as mean (±SD) for continuous variables, median and ranges for variables with a skewed distribution, and frequencies or percentages for categorical variables. Differences in mean values were assessed using t-tests, after log-transformation in case of skewed data and adjusted by Bonferroni correction for multiple testing. Differences in mean IMT and FMD between the various subgroups were also compared using t-tests. Categorical variables were compared using chi-square tests. Carotid and femoral IMT were stratified for age and risk factors for cardiovascular disease to assess the influence of these variables on outcome. To assess the influence of severity of hemophilia on atherogenesis, IMT, FMD and plaque data of severe and moderate hemophilia patients was combined and compared to controls.

**RESULTS**

The BMI was estimated prior to enrolment by using data on weight and length from the hemophilia treatment centers and was calculated after obtaining length and weight during the physical examination. After the initial selection of potentially eligible hemophilia patients meeting the BMI criteria, 2 patients were excluded because of the presence of HIV, 7 patients were excluded because of history of CVD and 27 patients did not want to participate due to either difficulty travelling to the study center, prior participation in recent studies or no interest in participation in studies.
A total of 205 study subjects were enrolled at the three study centers. Of these 205 subjects, 15 subjects (including hemophilia patients as well as controls) were excluded because they did not meet the BMI inclusion criteria (BMI ≥ 30 kg/m² or BMI ≤ 25 kg/m²) during the study visit. The remaining study population (n=190) consisted of 51 (26.8%) obese hemophilia A patients, 47 (24.7%) non-obese hemophilia A patients, 42 (22.1%) obese controls and 50 (26.3%) non-obese controls. Severity of hemophilia was equally distributed among the obese and normal weight patients (Table 1). Use of prophylactic or on demand treatment with factor VIII concentrate and the prevalence of hepatitis C infection was also not different between the two groups (Table 1). None of the patients had an inhibitor against factor VIII.

Table 1. Characteristics of hemophilia patients

<table>
<thead>
<tr>
<th>Type</th>
<th>Hemophilia BMI ≥ 30 kg/m² (N=51)</th>
<th>Hemophilia BMI ≤ 25 kg/m² (N=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe (FVIII &lt;1%)</td>
<td>17 (33%)</td>
<td>16 (34%)</td>
</tr>
<tr>
<td>Moderate (FVIII 1-5%)</td>
<td>8 (16%)</td>
<td>8 (17%)</td>
</tr>
<tr>
<td>Mild (FVIII 6-40%)</td>
<td>26 (51%)</td>
<td>23 (49%)</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophylactic</td>
<td>16 (31%)</td>
<td>12 (26%)</td>
</tr>
<tr>
<td>On demand</td>
<td>35 (69%)</td>
<td>35 (74%)</td>
</tr>
<tr>
<td>Hepatitis C infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>28 (55%)</td>
<td>25 (53%)</td>
</tr>
<tr>
<td>Current</td>
<td>10 (20%)</td>
<td>15 (32%)</td>
</tr>
<tr>
<td>In the past</td>
<td>13 (25%)</td>
<td>7 (15%)</td>
</tr>
</tbody>
</table>

Cardiovascular risk factors

Table 2 shows the presence of cardiovascular risk factors in hemophilia A patients and controls. As expected the subgroups were well matched for age and BMI. History of smoking in pack years was similar in all subgroups (p=1.00). Mean levels of systolic and diastolic blood pressure were significantly different between obese controls and non-obese controls (mean difference in systolic blood pressure 10 mmHg, p=0.003 and mean difference in diastolic blood pressure 8 mmHg, p=0.001). However, in hemophilia patients there was a higher prevalence of hypertension compared to the controls (43% and 25%, p=0.01). Fasting glucose levels were higher in obese compared to non-obese subjects in both hemophilia patients and in controls (p=0.014 and p=0.020, respectively).

Mean HDL levels were lower and triglyceride levels were higher in obese subjects compared to non-obese subjects, this difference reached statistical significance only in the controls and not in the hemophilia patients. Levels of total cholesterol and LDL cholesterol did not significantly differ between the groups. Dyslipidemia was also equally prevalent among hemophilia patients and controls (15% and 12%, p=0.50).
Table 2. Cardiovascular risk factors in hemophilia A patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Hemophilia BMI ≥ 30 kg/m² (N=51)</th>
<th>Hemophilia BMI ≤ 25 kg/m² (N=47)</th>
<th>Controls BMI ≥ 30 kg/m² (N=42)</th>
<th>Controls BMI ≤ 25 kg/m² (N=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.0 (13.7)</td>
<td>48.8 (13.9)</td>
<td>50.7 (12.0)</td>
<td>49.0 (13.6)</td>
</tr>
<tr>
<td>Weight (kg) a, b</td>
<td>107.8 (16.1)</td>
<td>75.4 (7.5)</td>
<td>109.1 (13.2)</td>
<td>77.1 (7.7)</td>
</tr>
<tr>
<td>Body mass Index (kg/m²) a, b</td>
<td>32.5 (30.1-50.2)</td>
<td>23.5 (18.7-25.0)</td>
<td>32.4 (30.0-50.2)</td>
<td>23.2 (18.5-25.0)</td>
</tr>
<tr>
<td>Waist circumference (cm) a, b</td>
<td>115.7 (12.2)</td>
<td>89.9 (6.6)</td>
<td>113.7 (11.4)</td>
<td>87.0 (6.9)</td>
</tr>
<tr>
<td>Smoking (pack years)</td>
<td>10 (6-55)</td>
<td>9 (0-45)</td>
<td>6.5 (0-47)</td>
<td>6.5 (0-50)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg) b</td>
<td>135.6 (15.8)</td>
<td>130.9 (16.5)</td>
<td>138.2 (14.8)</td>
<td>126.4 (9.8)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) b</td>
<td>83.0 (9.9)</td>
<td>79.1 (10.0)</td>
<td>84.4 (9.0)</td>
<td>76.2 (7.8)</td>
</tr>
<tr>
<td>Hypertension a, b, d</td>
<td>28 (55%)</td>
<td>14 (30%)</td>
<td>17 (41%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.4 (3.9-11.4)</td>
<td>5.2 (3.3-10.8)</td>
<td>5.3 (4.4-15.5)</td>
<td>5.1 (4.2-6.2)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>7 (14%)</td>
<td>1 (2%)</td>
<td>4 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.01 ± 1.01</td>
<td>5.09 ± 1.07</td>
<td>5.29 ± 1.05</td>
<td>5.20 ± 1.06</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l) b</td>
<td>1.22 ± 0.51</td>
<td>1.42 ± 0.42</td>
<td>1.21 ± 0.31</td>
<td>1.61 ± 0.64</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.17 ± 0.94</td>
<td>3.22 ± 0.96</td>
<td>3.40 ± 0.99</td>
<td>3.16 ± 0.96</td>
</tr>
<tr>
<td>Triglycerides (mmol/l) b</td>
<td>1.20 (0.34-4.89)</td>
<td>0.85 (0.27-2.50)</td>
<td>1.35 (0.26-4.49)</td>
<td>0.81 (0.28-2.19)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>10 (20%)</td>
<td>5 (11%)</td>
<td>7 (17%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Family history of premature CVD</td>
<td>11 (22%)</td>
<td>8 (17%)</td>
<td>15 (36%)</td>
<td>9 (18%)</td>
</tr>
</tbody>
</table>

Values are means ± SD or median and ranges for skewed data. P values by t-test (after log transformation if necessary) adjusted by Bonferroni correction for multiple testing or chi-square tests. CVD, cardiovascular disease. BMI, body mass index.

a P<0.05 for the comparison of obese hemophiliacs versus non-obese hemophiliacs
b P<0.05 for the comparison of obese controls versus non-obese controls
c P<0.05 for the comparison of obese hemophiliacs versus obese controls
d P<0.05 for the comparison of non-obese hemophiliacs versus non-obese controls

Carotid IMT

The mean carotid IMT in all hemophilia patients (IMT 0.74 ± 0.21 mm) was not different compared to all controls (IMT 0.72 ± 0.18 mm), mean difference 0.02 mm (95% CI -0.03-0.08 mm, p=0.45). Interestingly, mean carotid IMT was increased in obese subjects (IMT 0.77 ± 0.22 mm) compared to non-obese subjects (IMT 0.69 ± 0.16 mm), mean difference 0.07 mm (95% CI 0.02-0.13, p=0.008). When comparing obese hemophilia patients with obese controls, no difference in carotid IMT was apparent (IMT 0.78 ± 0.23 mm and 0.76 ± 0.22 mm, respectively, mean difference 0.02 mm, 95% CI -0.07-0.11, p=0.67) (Figure 1a). The mean carotid IMT was not different in severe and moderate hemophilia patients compared to controls, mean difference -0.03 mm (95% CI -0.09-0.04, p=0.44).
Femoral IMT

The mean femoral IMT in all hemophilia patients (IMT 0.87 ± 0.42 mm) was not different compared to all controls (IMT 0.85 ± 0.38), mean difference 0.02 mm; 95% CI -0.09-0.14 mm, p=0.72). The effect of obesity on femoral IMT is shown in figure 1b. The mean femoral IMT tended to be higher in obese subjects (IMT 0.90 ± 0.45 mm) compared to non-obese subjects (IMT 0.82 ± 0.33 mm), mean difference 0.08 mm (95% CI -0.03-0.20, p=0.16), although this difference was not statistically significant. The overall mean femoral IMT in obese hemophilia patients (IMT 0.92 ± 0.50 mm) was not different compared to obese controls (IMT 0.87 ± 0.40 mm), mean difference 0.06 mm (95%CI -0.13-0.25, p=0.55). Mean femoral IMT in severe and moderate hemophilia patients seemed lower when compared to controls, but did not reach statistical significance (mean difference in femoral IMT 0.06 mm, 95%CI -0.06-0.18, p=0.33).

Figure 1a. Mean intima media thickness of the carotid arteries

Carotid IMT

Figure 1b. Mean intima media thickness of the femoral arteries

Femoral IMT

IMT, intima media thickness
Adjusted IMT

Figure 2 shows the mean carotid and femoral IMT stratified by age. Overall a similar trend was observed in both hemophilia patients and in the controls, with a gradual increase in thickness of IMT of both the carotid and femoral artery with increasing age. Stratification for presence of cardiovascular risk factors, such as hypertension also showed no significant differences in mean carotid or femoral IMT.

**Figure 2a.** Mean carotid intima media thickness stratified for age

![Carotid Intima Media Thickness](image)

**Figure 2b.** Mean femoral intima media thickness stratified for age

![Femoral Intima Media Thickness](image)

IMT, intima media thickness; yrs, years
Plaques in carotid artery

The prevalence of atherosclerotic plaques (carotid IMT ≥ 1.3 mm) was assessed in all 6 segments of the carotid artery. Of the hemophilia patients, 33% had a plaque in one or more of the 6 segments of carotid artery, compared to 25% of the controls (p=0.25). The prevalence of plaques was similar in obese subjects compared to non-obese subjects (33% and 25%, respectively, p=0.23) and in obese hemophilia patients compared to obese controls (35% and 29% respectively, p=0.49). The presence of plaques in severe and moderate hemophilia patients was also similar compared to controls (27% and 25% respectively, p=0.79).

Flow mediated dilatation (FMD)

Table 3 shows the mean baseline brachial diameter and the mean peak post-occlusion artery diameter, which were comparable between hemophilia patients and controls (4.50 ± 0.67 mm and 4.47 ± 0.61 mm, p=0.75, and 4.70 ± 0.65 mm and 4.68 ± 0.60 mm, p=0.83, respectively). The mean FMD in hemophilia patients was also comparable to that of the control subjects (4.75% ± 2.84 and 4.93% ± 2.39, p=0.63). No effect of obesity on FMD could be detected in the subgroups. The FMD in obese subjects was comparable to the FMD in non-obese subjects (5.19% ± 2.79 and 4.51% ± 2.41 respectively, p=0.09). The FMD in obese hemophilia patients was also not different as compared to that of obese controls (4.84% ± 3.24 and 5.32% ± 2.37, p=0.45). When assessing the influence of severity of hemophilia on FMD, severe and moderate hemophilia patients had a similar FMD compared to controls (5.15% ± 3.26 and 4.93% ± 2.39, p=0.66).

Table 3. Baseline and post-occlusion hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Mean baseline brachial artery diameter (mm) ± SD</th>
<th>Mean peak brachial artery diameter (mm) ± SD</th>
<th>Mean FMD (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All controls</td>
<td>4.47 ± 0.60</td>
<td>4.68 ± 0.60</td>
<td>4.93 ± 2.40</td>
</tr>
<tr>
<td>All hemophiliacs</td>
<td>4.50 ± 0.70</td>
<td>4.70 ± 0.60</td>
<td>4.75 ± 2.80</td>
</tr>
<tr>
<td>Obese hemophiliacs</td>
<td>4.67 ± 0.70</td>
<td>4.88 ± 0.60</td>
<td>4.84 ± 3.20</td>
</tr>
<tr>
<td>Non-obese hemophiliacs</td>
<td>4.35 ± 0.70</td>
<td>4.55 ± 0.70</td>
<td>4.68 ± 2.50</td>
</tr>
<tr>
<td>Obese controls</td>
<td>4.45 ± 0.60</td>
<td>4.69 ± 0.60</td>
<td>5.32 ± 2.40</td>
</tr>
<tr>
<td>Non-obese controls</td>
<td>4.48 ± 0.60</td>
<td>4.68 ± 0.60</td>
<td>4.55 ± 2.40</td>
</tr>
<tr>
<td>All obese</td>
<td>4.53 ± 0.60</td>
<td>4.76 ± 0.60</td>
<td>5.10 ± 2.80</td>
</tr>
<tr>
<td>All non-obese controls</td>
<td>4.43 ± 0.70</td>
<td>4.63 ± 0.60</td>
<td>4.51 ± 2.40</td>
</tr>
</tbody>
</table>

SD, standard deviation

DISCUSSION

The present study indicates that obesity leads to an increased formation of carotid atherosclerosis, but that this process is not affected by a lifelong hypocoagulable state, namely hemophilia A. The overall mean carotid and femoral IMT, the prevalence of atherosclerotic plaques, and endothelial dysfunction, as measured by FMD, did not differ between obese hemophilia patients and obese
controls. Moreover, our study shows that also in obese hemophilia patients atherosclerotic plaques are prevalent, which predisposes them to future cardiovascular events.

Previously a protective effect of hemophilia on mortality due to ischemic heart disease was observed. Although the standardized mortality ratio varied (0.20 and 0.62), overall a 50% reduction in ischemic heart disease mortality was observed in hemophilia A patients compared to the general population. This beneficial effect of hemophilia on fatal artherothrombotic events could be the result of reduced thrombin formation. Thrombin is the key player in both fibrin formation and platelet activation. Thrombin cleaves fibrinogen to form fibrin, but can also trigger platelet activation through protease-activated receptors (PAR) 1 and 4. This may lead to the formation of thrombi and ultimately to vascular occlusion. Importantly, thrombin may also influence the process of atherosclerosis. Tissue factor and PARs are highly expressed in human atheroma and are induced in response to injury in animal models. In vitro, PAR activation induces leukocyte chemotaxis, smooth muscle cell proliferation and migration, which may lead to arterial remodeling and stenosis. In addition, coagulation factors and PARs are also involved in inflammatory responses and repair after injury. Although patients with hemophilia, who have a decreased thrombin formation, may be relatively protected from these atherosclerotic processes, we now show this is unlikely.

In previous studies, no clear association between hypocoagulability and IMT was shown. In 59 hemophilia A and B patients, carotid IMT was not different from controls (mean carotid IMT 0.76 mm (95% CI 0.71-0.80 mm) and 0.77 mm (95% CI 0.75- 0.80 mm) respectively), which was confirmed by Sartori and colleagues. In patients with severe type III von Willebrand disease similar results were found. When considering femoral IMT, no differences between hemophilia patients and controls were assessed. A small protective effect of hemophilia was, however, observed in patients with a moderate and severe type of hemophilia, whose mean femoral IMT was somewhat smaller compared to controls. Flow mediated dilatation, as a measure of endothelial dysfunction seemed to be impaired in hemophilia patients (3.8 ± 5.2% and 20.3 ± 13.0%, p=0.0001) compared to controls, but FMD in healthy controls was however remarkably high.

The previous studies had the same drawbacks. Patients were relatively young and had a low prevalence of cardiovascular risk factors. Therefore, a potential protective effect of hypocoagulability on atherosclerosis would have been difficult to detect. We, therefore, investigated hemophilia patients who all had a major risk factor for atherosclerosis, namely obesity. Strengths of this study include the careful selection of obese hemophilia patients and controls, as well as the use of validated surrogate markers for atherosclerosis. In addition, the ultrasound measurements were of high quality, which was confirmed by the low variation found between the repeated measurements. Furthermore the refusal to participate was very low in this population, since the majority of patients have a good relationship with hemophilia nurses and physicians who generally recruited these patients. The patients who did refuse due to health problems mostly had impaired mobility due to athropathy. Another potential limitation was the impossibility to detect effects of factor VIII levels less than 1% on atherosclerosis. Since almost all patients with severe
hemophilia receive regular prophylactic treatment, their phenotype turns to moderate. However, since there are no patients with lifelong factor VIII levels below 1% (due to treatment with factor VIII) this relationship is less relevant from a clinical point of view.

Our study has important clinical implications. We can conclude that hemophilia A patients with cardiovascular risk factors develop atherosclerosis to a similar extent as the general male population. This implies that detection and treatment of these risk factors in hemophilia patients is mandatory. Next, although the cardiovascular mortality is lower in hemophilia patients, the increasing life expectancy will lead to more cases of cardiovascular disease. The anticoagulant treatment of patients with hypocoagulability and consequently a higher bleeding risk is a major challenge.34

In conclusion, we show that patients with hemophilia and obesity, a major risk factor for atherosclerosis, have the same degree of subclinical atherosclerosis as obese control subjects. The reduced cardiovascular mortality of hemophilia patients can, therefore, most likely be attributed to protection against atherothrombosis.
REFERENCES


