Fibrin Formation and Degradation in Patients With Arteriosclerotic Disease

Thomas Herren, MD; Hans Stricker, MD; André Haeberli, PhD; Dai-Do Do, MD; P.W. Straub, MD

**Background** The blood coagulation cascade was reported to be activated in patients with arteriosclerotic disease of the lower limbs (peripheral arterial disease, PAD). There is more thrombin and fibrin formation compared with healthy control subjects. In many studies, however, the presence of arteriosclerotic disease had not been thoroughly ruled out in the control group. Therefore, markers of the activation of the blood coagulation cascade were measured in patients with PAD and in a carefully defined control group, both groups being subjected to an exercise test.

**Methods and Results** Twenty-two patients with angiographically documented PAD of grade II (Fontaine classification) and 13 control subjects in whom the presence of arteriosclerotic lesions was ruled out by noninvasive means in the carotid arteries, abdominal aorta, leg arteries, and coronary arteries took part in the study. Before and immediately after a treadmill stress test, the concentrations of prothrombin fragment F1+2 (F1+2), thrombin–antithrombin III complexes (TAT), fibrinopeptide A (FPA; this peptide was measured in spot urine also), and d-dimers were measured. Before exercise, the concentrations of F1+2 (1.0±0.6 versus 0.7±0.3 nmol/L), TAT (2.9±2.1 versus 1.9±0.8 μg/L), and d-dimers (318.2±270.1 versus 150.0±91.4 μg/L) were significantly higher in the patients with PAD compared with the healthy control subjects. FPA concentrations in plasma (1.9±1.0 versus 1.4±0.6 μg/L) and spot urine were not different, however. F1+2, FPA, and d-dimer concentrations correlated with the severity of the PAD as assessed by the ankle systolic blood pressure index (ABPI). The symptom-limited stress test did not lead to further activation of the blood coagulation cascade. However, concentrations of F1+2 (P<.001) and TAT (P<.01) after exercise correlated with the presence of ischemic changes in the stress-test ECG.

**Conclusions** There is evidence of enhanced thrombin formation in patients with PAD compared with an age- and sex-matched control group without clinical and sonographic evidence of arteriosclerosis. The thrombin formed, however, appears to be almost completely neutralized by antithrombin III. No direct evidence of fibrin formation was obtained, since the FPA concentrations were not different. In the patients with PAD, the higher concentrations of d-dimers are indicative of in vivo fibrinolysis. Thus, some fibrin formation must be postulated to occur in patients with arteriosclerosis.

(Circulation. 1994;90:2679-2686.)

**Key Words** • arteriosclerosis • fibrinolysis • thrombin

Thrombus formation in an artery, often associated with a fissured atheromatous plaque, may not only lead to occlusion of the vessel but also contribute to the development of the atheromatous lesion itself.2-4 Smith2 showed that fibrinogen and fibrin concentrations in cholesterol-rich advanced plaques are 10 times higher than in normal vessels. Analysis of the protein fraction isolated from complicated plaques indicated that fibrinogen II contributed the major proportion of fibrinogen-derived protein.5 Arteriosclerotic abdominal aneurysms contain cross-linked fibrin,6 among other proteins. Moreover, blood coagulation factors Xa and thrombin were shown to have mitogenic activity toward smooth muscle cells and fibroblasts in vitro and may therefore contribute to the pathogenesis of the disease.7,8 Several studies addressed the question of to what degree the coagulation cascade is activated in patients with arteriosclerotic disease of the lower limbs (peripheral arterial disease, PAD): Prothrombin fragment 1+2 (F1+2) and thrombin–antithrombin III complexes (TAT9-12) were consistently found to be elevated, indicating that thrombin is formed in these patients (in Reference 10, however, the difference compared with the control subjects was statistically not significant). Moreover, increased concentrations of d-dimers9,11-13 suggest that plasmin acted on cross-linked fibrin. However, the fibrinopeptide A (FPA) concentration in plasma, which is a direct marker of thrombin action on fibrinogen (Fig 1), was not consistently reported to be elevated.10,14 Depending on the severity of the PAD in the patients examined, various degrees of the activation of the coagulation cascade are observed.12 However, the discordant findings may be explained in part by the fact that the studies mentioned excluded the presence of arteriosclerotic disease in the control groups by history and physical examination only. In view of the high prevalence of arteriosclerotic disease, this may not be sufficient. Because the concentrations of F1+2, TAT, FPA, and d-dimers increase with age,15-17 selection of an age-matched control group is important. Therefore, the present study was undertaken to compare the degree of activation of the coagulation cascade and the fibrinolytic response in patients suffering from symptomatic, angiographically documented PAD (grade II according to Fontaine) with an age- and sex-matched control group. In the control group, the presence of arteriosclerotic lesions was determined to be unlikely by

Received May 9, 1994; revision accepted July 2, 1994.

From the Laboratory for Thrombosis Research (T.H., A.H., P.W.S.) and Division of Angiology (H.S., D.D.D.), Department of Medicine, University Hospital, Inselspital, Bern, Switzerland.


Correspondence to Thomas Herren, MD, The Cleveland Clinic Foundation, Center for Thrombosis and Vascular Biology, FF20, 9500 Euclid Ave, Cleveland, OH 44195.

© 1994 American Heart Association, Inc.
The study was approved by the Commission for Medical-Ethical Questions of the Medical Faculty of the University of Bern, Switzerland, and all patients and control subjects gave informed consent.

Patients

Twenty-two patients (46 to 80 years old; mean, 65 years) with symptomatic PAD grade II according to Fontaine were examined the day before percutaneous transluminal angioplasty (PTA) was performed on the culprit lesion. Six patients had undergone major vascular surgery ≥4 years before the study (peripheral or aortocoronary bypass operations), and 4 patients had undergone PTA before. All patients had an invasive radiological examination of the arteries of the lower extremities before PTA (Table 1): 13 had intravenous digital subtraction angiography, 4 had intra-arterial digital subtraction angiography, and 5 had intra-arterial angiography. In 19 of the 22 patients, the abdominal aorta could be well documented. According to the predominant localization of the arteriosclerotic lesions, the patients were classified as belonging to 1) the iliac artery type, 2) the femoral artery type, or 3) the mixed or generalized type.21 The systolic pressures of the ankle arteries were measured in duplicate with a Doppler ultrasound probe with the subjects in the resting state. For each leg, the ankle-brachial systolic blood pressure index (ABI) was calculated by dividing the ankle pressure by the highest upper-arm pressure value. The carotid arteries—ie, common, internal, and external carotid arteries on both sides—were examined for the presence of arteriosclerotic lesions by Doppler/duplex technique (Acuson 128 XP/5, 7.5-MHz linear transducer). The following criteria were applied (adapted from Reference 22): presence of arteriosclerotic plaques, bilateral disease, or stenoses. If a stenosis was present, it was graded as hemodynamically significant (>75%) or not significant (<75%).

Control Subjects

Twenty-five control subjects without evidence for arteriosclerotic disease in their histories and physical examinations (including bilateral palpation of the carotid, femoral, popliteal, posterior tibial, and dorsalis pedis arteries and auscultation of the mentioned arteries, including the abdominal aorta and excluding the pedal arteries, for audible bruits) and normal ECG during stress testing on the treadmill were screened by Doppler/duplex ultrasound examinations for the presence of arteriosclerotic lesions in the carotid arteries (see above). Moreover, the abdominal aorta was examined by ultrasound (Toshiba V SSA-100A, curved head; 3.75 MHz, type PVF 393 M) for the presence of arteriosclerotic plaques, dilatation, or stenosis. Subsequently, 12 subjects had to be excluded from the study because arteriosclerotic lesions were found in at least one location: 2 subjects had a positive treadmill stress test; 7 subjects had arteriosclerotic lesions in the abdominal aorta, including one aneurysm; and 6 had arteriosclerotic lesions in the carotid arteries. The age of the remaining 13 control subjects (48 to 76 years; mean, 63 years) was not different from the patient group. The systolic pressures of the ankle arteries were measured in duplicate with a Doppler ultrasound probe with the subjects in the resting state, and ABI was calculated as described above.

The hospitalized patients and the age- and sex-matched control subjects performed an incremental exercise stress test on a treadmill (10% uphill; Woodway GmbH), which was symptom limited for the patients. The control subjects were instructed to walk as fast as possible. An ECG was continuously recorded, and a representative sample was printed.

### Table 1. Results of Radiological Examinations in Patient Group (n=22)

<table>
<thead>
<tr>
<th>Carotid arteries (ultrasound examination), N (%)</th>
<th>Presence of plaques</th>
<th>14/19 (74)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral disease</td>
<td>10/19 (53)</td>
<td></td>
</tr>
<tr>
<td>Stenosis of CCA</td>
<td>&lt;75%</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Stenosis of ICA</td>
<td>&lt;75%</td>
<td>2/19 (11)</td>
</tr>
<tr>
<td>&gt;75%</td>
<td>2/19 (11)</td>
<td></td>
</tr>
<tr>
<td>Abdominal aorta (angiography), N (%)</td>
<td>Presence of plaques</td>
<td>17/19 (90)</td>
</tr>
<tr>
<td>Dilatation of AA</td>
<td>1/19 (5)</td>
<td></td>
</tr>
<tr>
<td>Stenosis of AA</td>
<td>1/19 (5)</td>
<td></td>
</tr>
<tr>
<td>Angiography of lower extremities, N (%)</td>
<td>Iliac artery type</td>
<td>7/22 (32)</td>
</tr>
<tr>
<td>Femoral artery type</td>
<td>3/22 (13)</td>
<td></td>
</tr>
<tr>
<td>Mixed or generalized type</td>
<td>12/22 (55)</td>
<td></td>
</tr>
</tbody>
</table>

CCA indicates common carotid artery; ICA, internal carotid artery; ECA, external carotid artery; and AA, abdominal aorta.

*Three patients were not examined.

†Numbers in parentheses indicate percentages.

‡Classification according to Kappert.21
before each increment and analyzed. A horizontal or
downsloping ST-segment depression of ≥0.10 mV 60 milli-
seconds after the J-point was considered to be abnormal.23 If
the patient complained of chest pain or if ST-segment depressions
were present on the ECG, the stress test was immediately
terminated.

Laboratory Investigations

Before exercise, patients and control subjects gave a 10-mL
sample of spontaneously voided urine, in which the concen-
tration of creatinine was measured. Nine hundred microliters
of the urine was mixed with 100 μL of an aqueous solution of
heparin-aprotinin (stock solution containing 1000 U hepa-
rin/mL and 1000 U aprotinin/mL plus 0.02% sodium azide) for
determination of FPA (radioimmunoassay [RIA], see below):
Bentonite adsorption was performed only if blood was present
in the urine samples.24 All urine samples were tested for the
determination of FPA (radioimmunoassay [RIA], see below):
Bentonite adsorption was performed only if blood was present
in the urine samples.24 All urine samples were tested for the
presence of blood with MultiStix test strips (Boehringer).

Blood samples were collected before and within 3 minutes
after exercise, between 4 and 6 PM. Blood was collected from
supine subjects with a clean venipuncture (20-gauge needle)
from an antecubital vein under controlled venous stasis of 60
mm Hg by use of the Sarstedt system (Sarstedt Nümbrecht) in
the following sequence.

1. Blood (9 mL) was added to 1 mL of CTAEDPACK
anticoagulant (stock solution containing 25 mL of citrate-
theophylline-adenosine-dipyridamide [Becton-Dickinson] plus
5 mg of phenyl-prolyl-arginine-chloromethylketone [Calbio-
chem], giving a PPACK concentration of 400 μmol/L) for
measurement of Fl+2 (ELISA Enzygnost F1+2 by Behring25),
TAT complexes (ELISA Enzygnost TAT by Behring25), and
FPA (RIA reagents supplied by Imco27).

FPA was determined in PPACK-inhibited plasma samples
by the RIA mentioned above using polyclonal antibodies.
Cross-reacting fibrinogen was eliminated by bentonite adsorp-
tion. Free antigen was separated from bound antigen by use of
a second goat anti-rabbit antibody (Immunobeads, Bio-Rad
Laboratories).

2. Blood (9 mL) was added to 1 mL of 0.106 mol/L trisodium
citrate for assessment of activated partial thromboplastin time
(aPTT) by use of Pathromtin (Behring), fibrinogen (Clauss
method28), and euglobulin clot lysis time (ECLT). D-Dimer
concentrations were measured with an ELISA by Kabi Diag-
nostics (CoaZila D-dimer29).

3. Blood (2 mL) was added to two drops of EDTA-potassium
fluoride (Boehringer) for determination of lactate levels.

4. Blood (4.5 mL) was added to 5 mg of dry EDTA for
leukocyte and platelet count and hemoglobin determination
(Coulter Counter S+, Coulter Electronics). Duplicate sam-
plies were centrifuged at 12 000g for 5 minutes (Clay-Adams
Autocrit centrifuge, Becton Dickinson), and the hematocrit
value was determined immediately after blood sampling.

5. Blood (4.5 mL) was added to 0.5 mL heparin-NH2 for
determination of preexercise creatinine and urea concentra-
tions and preexercise and postexercise concentrations of albu-
mun and total protein in plasma.

6. Blood (5 mL) was allowed to clot for 1 hour. In the
supernatant serum, the total cholesterol concentration was
measured.

7. Blood (1.8 mL) was added to 0.2 mL of 0.106 mol/L
trisodium citrate for measurement of the blood sedimentation
rate (before exercise only). Mean blood sedimentation rate
was 17 for the patients and 10 for the control subjects.

All venipunctures were performed by the same investigator.
Immediately after the blood was sampled, tubes 1 through 3
were put on melting crushed ice for a minimum of 10 minutes.
The tubes were centrifuged at 4°C for 20 minutes at 2000g.
Multiple aliquots of plasma were then snap-frozen in liquid
nitrogen and stored at −70°C until analysis.

The changes in plasma volume were calculated according to
Dill and Costill30 and were −3.3±3.9% in the patient group
and −9.2±5.2% in the control group. Alternatively, using the
plasma protein method, these values were −2.5±4.3% and
−6.3±3.3%, respectively. Therefore, postexercise concentra-
tions of high-molecular-weight proteins (fibrinogen, F1+2,
TAT, d-dimers) were corrected for the hemococoncentration
occurring during exercise. Concentrations of low-molecular-
weight proteins (FPA) and lactate were not corrected for the
decrease in plasma volume, since it is unlikely that their
concentrations were affected by the fluid shift.

Statistical Analysis

Preexercise and postexercise values were analyzed by Stu-
dent’s t test for paired data (P is given for two-tailed analysis).
Concentrations between patients and control subjects were
compared by the t test for independent samples and separate
variances, and the nonparametric Mann-Whitney U test was
used where appropriate. When the variances between patients
and control subjects differed considerably, we refrained from
using the nonparametric test. Correlations were examined by
least-squares regression. Differences were considered signifi-
cant at P<.05. Values are given as mean±SD.

Results

The patients and control subjects were matched for age and sex (Table 2), and there were no significant
differences for body mass index and mean arterial blood
depression. As expected, the vascular risk factor profile
differed: Total cholesterol concentration was higher in the
patient group. The percentage of current smokers was higher in the patient group (50% versus 8%), and
the fibrinogen concentration was elevated in the patient
group, but not significantly different from the control
group. Although the percentages of hypertensive and
diabetic patients were higher in the patient group, the
differences were not statistically significant. Because of
the presence of PAD, more patients received acetylsal-
icyl acid (Table 2) compared with the healthy control
subjects. The ABPI was lower in the patient group, and
the patients walked a shorter distance than the control
subjects during stress testing. Eight patients had a
history of angina pectoris or myocardial infarction, and
three additional patients (total of five) had ST-segment
depression in the stress-test ECG.

Shortening of the aPTT and ECLT were measured after
exercise (Table 3) in both groups, but shortening of the
ECLT in the control group was found to be not
statistically significant. The aPTT and ECLT before
exercise were shorter in the control group. Blood plate-
let and leukocyte counts increased in both groups
during exercise. Hemoconcentration was more
pronounced in the control subjects compared with the
patients (increases in the centrifuged hematocrit value
and hemoglobin concentration), corresponding to
decreases in plasma volume of −9.2% and −3.3%, respec-
tively (see "Methods"). Hemoconcentration was also
documented by the increase in total protein concentra-
tion in both groups. Fibrinogen concentration remained
unchanged. The lactate concentrations increased signifi-
cantly in both groups and reached similar values after
exercise (2.8 and 3.1 mmol/L, respectively).

The concentrations of F1+2, TAT, FPA, and d-di-
mers did not change during exercise (Table 4). How-
ever, the concentrations before exercise differed be-
tween the two groups (Table 4, Fig 2): The concentrations of F1+2 and TAT were significantly
higher in the patient compared with the control group.
Table 2. Study Population

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>13</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>64.8±8.0</td>
<td>63.0±7.5</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>14/8</td>
<td>9/4</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.1±3.7</td>
<td>24.7±3.2</td>
<td>NS</td>
<td>22.7 (M), 22.4 (F)</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>108.8±11.8</td>
<td>114.4±14.9</td>
<td>NS</td>
<td>&lt;110</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/L</td>
<td>107.7±7.5</td>
<td>101.0±2.8</td>
<td>NS</td>
<td>59-116 (M), 45-102 (F)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.9±1.1</td>
<td>6.0±0.9</td>
<td>&lt;.05</td>
<td>&lt;5.2</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>3.2±0.8</td>
<td>2.8±0.6</td>
<td>.07</td>
<td>1.5-3.5</td>
</tr>
<tr>
<td>Current users of acetylsalicylic acid, N (%)</td>
<td>15/22 (68)</td>
<td>1/13 (8)</td>
<td>&lt;.01</td>
<td>...</td>
</tr>
<tr>
<td>Ankle/brachial systolic pressure index</td>
<td>0.7±0.2</td>
<td>1.1±0.1</td>
<td>&lt;.001</td>
<td>&gt;0.92</td>
</tr>
<tr>
<td>Walking distance on treadmill, m</td>
<td>271.3±149.4</td>
<td>593.1±74.5</td>
<td>&lt;.001</td>
<td>...</td>
</tr>
<tr>
<td>History of myocardial infarction or angina pectoris, N (%)</td>
<td>8/22 (36)</td>
<td>0/13 (0)</td>
<td>&lt;.05</td>
<td>...</td>
</tr>
<tr>
<td>Vascular risk factors, * N (%)</td>
<td>Current smokers</td>
<td>11/22 (50)</td>
<td>1/13 (8)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11/22 (50)</td>
<td>5/13 (38)</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>8/22 (36)</td>
<td>1/13 (8)</td>
<td>NS</td>
<td>...</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2/22 (9)</td>
<td>1/13 (8)</td>
<td>NS</td>
<td>...</td>
</tr>
</tbody>
</table>

*Anamnestic facts.

There was a significant correlation between the preexercise F1+2 and TAT concentrations (Fig 3). Six of 22 patients had TAT concentrations that were higher than the mean concentration plus 2 SD (3.36 μg/L) of the control subjects. The FPA concentrations, although higher in the patient group, were not significantly different, as was the FPA excretion in the spot urine (patients, 3.50±2.41 versus control subjects, 2.48±1.35 ng FPA/mg creatinine; P=.1). However, the d-dimer concentration in the patient group was higher than in the control group (Fig 2), and 8 of 22 patients (36%) had d-dimer concentrations ≥350 μg/L.

Regression analysis revealed that in the patient group, the ABI correlated with the concentrations of F1+2 (r=.43, P<.05), FPA (r=.58, P<.01), and d-dimers (r=.61, P<.01) measured before exercise. Moreover, patients with presence of ST-segment depression in the stress-test ECG tended to have higher mean F1+2 and TAT concentrations after exercise (F1+2, r=.56, P<.001; TAT, r=.46, P<.01).

As shown in Fig 4, there was no correlation between the type of peripheral atherosclerotic disease (iliac or proximal, femoral, and mixed or generalized types21) and TAT concentrations (P=.82). TAT concentrations were not correlated with the presence of atherosclerotic lesions in the carotid arteries (Fig 4, P=.72). Moreover, significant correlations were found between age and both F1+2 and FPA concentrations when the two groups were examined together. F1+2, FPA, and d-dimer but not TAT concentrations were each significantly correlated with the creatinine and/or urea concentrations. No correlation existed between the aPTT before exercise and the examined immunological coagulation parameters.

To address the question of whether the concentrations of the examined immunological markers of the activated coagulation cascade changed with time, a subset of three patients was reexamined 4 weeks after the first examination. The mean F1+2 (1.43 versus 2.01 nmol/L), TAT (6.77 versus 9.47 ng/mL), and FPA (2.44 versus 1.24 ng/mL) did not vary substantially.

Discussion

The most important finding of the present study is that the blood coagulation cascade is activated to a higher degree in patients with symptomatic PAD (grade II according to Fontaine) than in control subjects of the same age and sex in whom the presence of arteriosclerotic disease had been ruled out noninvasively directly (Doppler/duplex scanning) in the carotid arteries and in the abdominal aorta and indirectly (absence of symptoms, measurement of the ABI) in the leg arteries and in the coronary circulation (normal treadmill stress-test ECG). These findings concur with other reports showing enhanced concentrations of F1+2, TAT, and d-dimers but not significantly different FPA concentrations in patients with PAD compared with definitely less strictly defined control subjects.

In the control group of the present study, 48% of the screened subjects had to be excluded because of the presence of arteriosclerotic lesions (see "Methods"). The prevalence of 24% of occult arteriosclerotic carotid artery lesions in asymptomatic subjects having vascular risk factors (Table 2) concurs with the findings reported by Prati et al.32 (Even with sophisticated noninvasive techniques, the diagnosis of arteriosclerosis can be difficult, and there are no generally agreed-on definitions of the level at which normal ends and disease begins.33 In this study, a slight increase in intimal thickness was not considered to represent an arteriosclerotic lesion [see "Methods" and References 33 and 34]. Even though the control population was screened
carefully for the presence of arteriosclerotic disease, there may be lesions that escaped our detection.) The prevalence of abdominal aortic aneurysms was reported to be 5.4% in men 65 to 74 years old, which is in good agreement with the 4% found in this study. We detected no subject with PAD in the lower limbs in the control group. The prevalence of large-vessel PAD is estimated to be 12% in an asymptomatic population. It cannot be excluded that small lesions escaped detection when the ABPI was used as a screening test. However, the observed differences in the concentrations of F1+2, TAT, FPA, and D-dimers (Fig 2) were no longer present when all screened subjects were included in the analysis (results not shown). This finding underscores the importance of excluding the presence of arteriosclerosis in a control population.

The differences in the examined parameters in patients with PAD versus control subjects are small but significant (Fig 2). Compared with other studies, the patients had less severe PAD as assessed by the ABPI, which may have minimized the difference. The present study confirms earlier reports that the extent of activation of the coagulation cascade, ie, the concentrations of F1+2, FPA, and D-dimers, is correlated with the severity of the PAD.

In the patients, concentrations of F1+2 and TAT in the resting state were higher than in the control subjects (Fig 2). This finding supports the hypothesis that thrombin is formed in patients with arteriosclerotic disease. However, we found no direct evidence for fibrin formation, since the FPA concentration in the plasma (Table 4) was not significantly different in the two groups examined. We determined the FPA concentration in a spot urine sample also, because the plasma half-life of FPA is only approximately 4 minutes, and its concentration in the urine may more accurately reflect intermittent fibrin formation. The concentration in the patient group was found to be elevated but not significantly different from the control group (patients, 3.5 versus control subjects, 2.5 ng FPA/mg creatinine; see “Results”). The finding that FPA concentrations measured in plasma and urine were not significantly differ-

---

Table 3. aPTT, ECLT, Blood Platelet and Leukocyte Counts, Hematocrit, Hemoglobin, Fibrinogen, Total Protein, and Lactate Concentrations Before and After Exercise

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>Normal Value</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT, s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>39.0±3.5</td>
<td>34.7±3.7</td>
<td>30-40</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>After</td>
<td>34.9±3.0‡</td>
<td>32.6±3.7†</td>
<td>30-40</td>
<td>NS</td>
</tr>
<tr>
<td>ECLT, min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>269.7±50.8</td>
<td>202.9±74.4</td>
<td>&gt;120</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>After</td>
<td>165.7±87.9§</td>
<td>175.1±93.4</td>
<td>&gt;120</td>
<td>NS</td>
</tr>
<tr>
<td>Blood platelet count, ×10⁶/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>251.3±61.9</td>
<td>244.8±57.3</td>
<td>125-320</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>270.6±71.3‡</td>
<td>267.4±63.1§</td>
<td>125-320</td>
<td>NS</td>
</tr>
<tr>
<td>Leukocyte count, ×10⁶/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.8±1.8</td>
<td>7.2±1.3</td>
<td>3.2-9.0</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>9.5±2.0§</td>
<td>9.1±2.0§</td>
<td>3.2-9.0</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>43.4±3.8</td>
<td>42.6±4.1</td>
<td>42-52</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>43.8±3.7</td>
<td>45.1±3.6†</td>
<td>42-52</td>
<td>NS</td>
</tr>
<tr>
<td>Hemoglobin concentration, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>142±14</td>
<td>148±13</td>
<td>135-168</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>146±14§</td>
<td>156±14§</td>
<td>135-168</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein concentration, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>76.2±4.0</td>
<td>71.3±2.7</td>
<td>59.0-80.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>After</td>
<td>78.2±3.5†</td>
<td>76.1±4.0§</td>
<td>59.0-80.0</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen concentration, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>3.2±0.8</td>
<td>2.8±0.6</td>
<td>1.5-3.5</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>3.2±0.9</td>
<td>2.7±0.5</td>
<td>1.5-3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Blood lactate concentration, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.9±0.3</td>
<td>1.2±0.5</td>
<td>0.63-2.44</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>2.8±2.1§</td>
<td>3.1±2.7†</td>
<td>0.63-2.44</td>
<td>NS</td>
</tr>
</tbody>
</table>

aPTT indicates activated partial thromboplastin time; ECLT, euglobulin clot lysis time. Values are mean±SD.

*By t test for unpaired data.
†P<.05; ‡P<.01; §P<.001 (paired t test).
TABLE 4. Concentrations of F1+2, TAT, FPA, and D-Dimers Before and After Exercise

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2, nmol/L</td>
<td>1.0±0.6</td>
<td>0.7±0.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Before</td>
<td>1.1±0.6</td>
<td>0.6±0.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>After</td>
<td>2.9±2.1</td>
<td>1.9±0.8</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
<td>2.5±1.7</td>
<td>1.8±0.5</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>FPA, ng/mL</td>
<td>1.9±1.0</td>
<td>1.4±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Before</td>
<td>1.7±1.1</td>
<td>1.6±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>318.2±270.1</td>
<td>150.0±91.4</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>D-Dimers, ng/mL</td>
<td>293.2±199.7</td>
<td>158.8±92.4</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

F1+2 indicates prothrombin fragment F1+2; TAT, thrombin-antithrombin III complex; and FPA, fibrinopeptide A. Values are mean±SD.

*By t test for unpaired data.

ent between patients and control subjects is in agree-
ment with recently published studies10,11 but differs somewhat from older studies,14,37,38 which may be attributed in part to methodological differences. While we used a highly specific thrombin inhibitor as an anticoagu-
lant (PPACK39) and processed the blood without delay to minimize in vitro artifacts, Donaldson et al.38 used an anticoagulant that may not completely inhibit thrombin and processed the blood within 3 hours. Moreover, the observed correlation between the FPA concentration and the severity of the disease suggests that FPA generation may be seen in patients with more severe forms of PAD, which was recently confirmed.12 Also, patients with aortic abdominal aneurysms were reported to have high FPA concentrations in plasma.14

In the patient group, there was only one patient who had a dilatation of the abdominal aorta (Table 1), in whom the FPA concentration in plasma was 4.0 ng/mL.

Despite the results of the FPA determinations dis-
cussed above, some fibrin formation must have occurred in the patient group, because the D-dimer concentration was higher than in the control group. D-Dimers are indicative of fibrinolysis of cross-linked fibrin.20 Because the half-life of D-dimers is on the order of 8 hours,20 this parameter may be a more reliable indicator of in vivo fibrin formation (and subsequent catabolism) than FPA measured in plasma or in spot urine samples. Thus, even though part of the thrombin formed was presumably inactivated by antithrombin III (high TAT concentrations), fibrin formation occurred, and it may be postulated that the quenching capacity of antithrombin III has been surpassed locally or momentarily.

These findings are relevant with respect to the cutoff values chosen for the use of TAT and D-dimer assays in the diagnosis of pulmonary embolism. Demers et al.41 suggest concentrations of 3.5 and 300 ng/mL, respectively. However, in the present study, 5 and 10 patients, respectively, out of a group of 22 would have fulfilled the criteria for diagnosing pulmonary embolism if these cutoff values were used. Therefore, in patients with significant arteriosclerotic disease, these tests should be used cautiously.

Confirmation of the correlation between increasing age and the plasma concentrations of F1+2 and FPA15 was obtained in this study when patients and control groups were examined together but not when they were analyzed separately. Moreover, the F1+2 concentration measured in the control group (Table 4) is not different from the value obtained in a considerably younger group of healthy individuals by use of the same assay.20 Thus, age per se does not seem to influence the concentration of F1+2. It may be that occult arteriosclerotic lesions are responsible for the measured age-dependent increase of its concentration.

The observed correlation between the F1+2, FPA, and D-dimer concentrations and the degree of renal insufficiency may be because the prevalence and extent
of atherosclerotic disease are higher in patients with renal insufficiency compared with the general population.42 However, clearance of FPA25 and, to a lesser extent, F1+243 may be lower in patients with renal insufficiency. The plasma concentrations of TAT complexes, which are cleared from circulation by a common serpin receptor on hepatocyte membranes (half-life, 2 minutes44), as well as D-dimers, which are catabolized primarily by the liver because of their molecular weight of 190 000,45 are not influenced by decreased renal function. Thus, high TAT and D-dimer concentrations in particular may correspond to increased thrombin formation and reactive fibrinolysis, respectively,45 in these patients as well.

Patients suffering from advanced stages of PAD with high F1+2 and TAT concentrations, indicative of a prethrombotic state,46 may benefit most from anticoagulant therapy,47,48 especially if they have comitant signs of ischemic heart disease: In the present study, patients with a pathological stress-test ECG tended to have higher F1+2 and TAT concentrations (see "Results" and Reference 17). It is of interest to note that half of the patients had either a history of angina pectoris and myocardial infarction and/or a pathological stress-test ECG. This finding concurs with a recent report by Ogren et al49 in which the presence of PAD was associated with an increased cardiac event rate. Also, high D-dimer concentrations were reported to be a predictor of progression of PAD and of future coronary events.14,50

Because endothelial cells cultured under hypoxic and acidic conditions during 48 hours efficiently activate factor X,51 thus initiating cell surface assembly of a prothrombinase complex,3 we addressed the question of whether increasing ischemia during a treadmill stress test would increase the thrombin formation in the patient group. However, the short treadmill test performed by our study subjects was not sufficient to modulate the activation of the blood coagulation cascade (Table 4).

In conclusion, compared with a carefully defined control group without signs of atherosclerosis, patients with PAD had increased F1+2 and TAT concentrations, indicative of thrombin formation. The finding of a simultaneously increased D-dimer concentration is an indirect argument for the occurrence fibrin formation. The correlations of F1+2, FPA, and D-dimer concentrations with the severity of the PAD as assessed by the ABPI and of the postexercise concentrations of F1+2 and TAT with the presence of cardiac ischemia in the ECG may be of importance for the management of these patients.

Acknowledgments

The authors thank D. Spina for expert technical assistance, the technicians of the angiology laboratory for measurement of ankle systolic pressures, and Dr P. Ruisi from the Department of Radiology for performing the ultrasound examinations of the abdominal aorta. We also thank Dr E. Gardiner for critical review of the manuscript.

References


Fig 4. Individual concentrations of thrombin-antithrombin III (TAT) complexes before exercise in patients (n=22) and control subjects (n=13). Six patients, but no control subjects, have TAT concentrations >3.36 μg/L (corresponding to the mean+2 SD TAT concentration of the control subjects). No correlation between the TAT concentration and the extent or localization of the arteriosclerotic disease could be documented (P=.82). Iliac artery type of arteriosclerosis ( ), femoral artery type ( ), mixed type ( ), and control subjects ( ). Filled symbols denote the presence of atherosclerotic lesions in the carotid arteries. In three patients ( ), the latter were not examined.


Fibrin formation and degradation in patients with arteriosclerotic disease.

T Herren, H Stricker, A Haeberli, D D Do and P W Straub

Circulation. 1994;90:2679-2686
doi: 10.1161/01.CIR.90.6.2679

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/90/6/2679

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/