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.

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**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. Smith showed that fibrinogen and fibrinogen 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 contributed the main protein of fibrinogen-derived protein. Arteriosclerotic abdominal aneurysms contain cross-linked fibrin, 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. Several studies addressed the question of 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 (TAT1-12) were consistently found to be elevated, indicating that thrombin is formed in these patients (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. Depending on the severity of the PAD in the patients examined, various degrees of the activation of the coagulation cascade are observed. 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

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(1) clinical examination, (2) noninvasive radiological documentation, \(^{16,19}\) and (3) a treadmill stress test. The last was performed in consideration of the fact that the concentrations of F1 + 2 and TAT are also elevated in patients with coronary atherosclerosis. \(^{17}\) To address the question of whether the extent of activation of the coagulation cascade increased with physical exercise, \(^{20}\) the study subjects were examined both before and after a stress test on a treadmill.

**Methods**

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 atherosclerotic 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 (ABPI) 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 ABPI 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.

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

| Carotid arteries (ultrasound examination),* N (%) | Presence of plaques | 14/19 (74)*\(\text{t}\) |
| Bilateral disease | 10/19 (53) |
| Stenosis of CCA | 0/0 |
| ＜75% | 0/0 |
| ＞75% | 2/19 (11) |
| Stenosis of ICA | 2/19 (11) |
| ＜75% | 0/0 |
| ＞75% | 2/19 (11) |
| Abdominal aorta (angiography),* N (%) | Presence of plaques | 17/19 (90) |
| Dilatation of AA | 1/19 (5) |
| Stenosis of AA | 1/19 (5) |
| Angiography of lower extremities,† N (%) | Iliac artery type | 7/22 (32) |
| Femoral artery type | 3/22 (13) |
| Mixed or generalized type | 12/22 (55) |

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 milliseconds 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 concentration 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 heparin/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 presence of blood with Multistix test strips (Bohringer).

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 CTADPPACK anticoagulant (stock solution containing 25 mL of citrate-theophylline-adenosine-dipridamol [Becton-Dickinson] plus 5 mg of phenyl-prolyl-arginine-chloromethylketone [Calbiochem], giving a PPACK concentration of 400 μmol/L) for measurement of F1+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 adsorption. 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 Diagnostica (Coaliza D-dimer²⁹).

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

4. Blood (4.5 mL) was added to 5 mL of dry EDTA for leukocyte and platelet count and hemoglobin determination (Coulter Counter S+, Coulter Electronics). Duplicate samples 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-NH₄ for determination of preexercise creatinine and urea concentrations and preexercise and postexercise concentrations of albumin 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 concentrations of high-molecular-weight proteins (fibrinogen, F1+2, TAT, D-dimers) were corrected for the hemoconcentration 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 Student'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 significant 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 pressure. 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 acetylsalicylic 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 platelet 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 −3.5%, respectively (see "Methods"). Hemoconcentration was also documented by the increase in total protein concentration in both groups. Fibrinogen concentration remained unchanged. The lactate concentrations increased significantly 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-dimers did not change during exercise (Table 4). However, the concentrations before exercise differed between 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.
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 ABPI 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 types) 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 ABPI) 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. (Even with sophisticated noninvasive techniques, the diagnosis of atherosclerosis can be difficult, and there are no generally agreed-on definitions of the level at which normal ends and disease begins. 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>
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<th>Patients</th>
<th>Control Subjects</th>
<th>Normal Value</th>
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<tr>
<td>aPTT, s</td>
<td></td>
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<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>
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<td>ECLT, min</td>
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<td></td>
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<tr>
<td>Before</td>
<td>269.7±50.8</td>
<td>202.9±74.4</td>
<td>&gt;120</td>
<td>&lt;.05</td>
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<tr>
<td>After</td>
<td>165.7±87.9§</td>
<td>175.1±93.4</td>
<td>&gt;120</td>
<td>NS</td>
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<td>Blood platelet count, ×10⁶/L</td>
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<tr>
<td>Before</td>
<td>251.3±61.9</td>
<td>244.8±57.3</td>
<td>125-320</td>
<td>NS</td>
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<tr>
<td>After</td>
<td>270.6±71.3§</td>
<td>267.4±63.1§</td>
<td>125-320</td>
<td>NS</td>
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<tr>
<td>Leukocyte count, ×10⁶/L</td>
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<tr>
<td>Before</td>
<td>7.8±1.8</td>
<td>7.2±1.3</td>
<td>3.2-9.0</td>
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<tr>
<td>After</td>
<td>9.5±2.0§</td>
<td>9.1±2.0§</td>
<td>3.2-9.0</td>
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<td>Hematocrit, %</td>
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<td>Before</td>
<td>43.4±3.8</td>
<td>42.6±4.1</td>
<td>42-52</td>
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<tr>
<td>After</td>
<td>43.8±3.7</td>
<td>45.1±3.6†</td>
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<td>Hemoglobin concentration, g/L</td>
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<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>
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<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>
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<td>Fibrinogen concentration, g/L</td>
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<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>
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<tr>
<td>Blood lactate concentration, mmol/L</td>
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<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).

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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>
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</thead>
<tbody>
<tr>
<td>F1+2, nmol/mL</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.0±0.6</td>
<td>0.7±0.3</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>After</td>
<td>1.1±0.6</td>
<td>0.6±0.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>TAT, ng/mL</td>
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<td></td>
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<tr>
<td>Before</td>
<td>2.9±2.1</td>
<td>1.9±0.8</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>After</td>
<td>2.8±1.7</td>
<td>1.8±0.5</td>
<td>&lt;.05</td>
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<tr>
<td>FPA, ng/mL</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.9±1.0</td>
<td>1.4±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>1.7±1.1</td>
<td>1.6±0.5</td>
<td>NS</td>
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<tr>
<td>d-Dimers, ng/mL</td>
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</tr>
<tr>
<td>Before</td>
<td>318.2±270.1</td>
<td>150.0±91.4</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>After</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 agreement with recently published studies but differs somewhat from older studies, which may be attributed in part to methodological differences. While we used a highly specific thrombin inhibitor as an anticoagulant (PPACK) and processed the blood without delay to minimize in vitro artifacts, Donaldson et al. 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. Also, patients with aortic abdominal aneurysms were reported to have high FPA concentrations in plasma. 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 discussed 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. Because the half-life of d-dimers is on the order of 8 hours, 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. 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 FPA 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. 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

![Fig 2. Box plots showing medians and 5th, 10th, 25th, 75th, 90th, and 95th percentiles of data of prothrombin fragment F1+2 (F1+2), thrombin-antithrombin III (TAT), fibrinopeptide A (FPA), and d-dimer concentrations in patients and control subjects. The dashed line in each box shows the mean value of the data (see Table 4).](image)
of atherosclerotic disease are higher in patients with renal insufficiency compared with the general population.43 However, clearance of FPA28 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 concomitant 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.50,51

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 arteriosclerosis, 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 ABI 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.

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