Impairment of the Diurnal Fibrinolytic Response in Man
EFFECTS OF AGING, TYPE IV HYPERLIPOPROTEINEMIA, AND CORONARY ARTERY DISEASE

By Douglas R. Rosing, David R. Redwood, Pieter Brakman, Tage Aitrup, and Stephen E. Epstein

ABSTRACT

Diurnal patterns of plasma euglobulin fibrinolytic activity, estimated by the fibrin plate method, were determined in groups of young normal and older normal subjects, in subjects with type IV hyperlipoproteinemia, and in subjects with coronary artery disease who had normal lipid profiles. The marked diurnal increases in fibrinolytic activity observed in the young normal subjects were significantly reduced in a large percent of the older normal subjects and in most of the subjects with coronary artery disease or type IV hyperlipoproteinemia. Although not conclusive, these findings were compatible with the hypothesis that an impairment in the responsiveness of the fibrinolytic system may be related to the development of coronary artery disease.

KEY WORDS  plasminogen activators stress euglobulin
triglyceride fraction fibrinogen atherosclerosis

The mechanisms responsible for the development of atherosclerotic vascular disease are still poorly understood. A concept currently under debate postulates that, during the course of tissue injury and repair, there is a dynamic balance between the deposition and the dissolution of fibrin and that an imbalance in this system, caused by impaired fibrinolysis, may lead to the persistence of fibrin deposits at sites of luminal injury, the incorporation of the fibrin into the vessel wall, and, ultimately, the development of degenerative atherosclerotic changes (1-3). Therefore, attempts have been made to determine a possible relation between impaired blood fibrinolysis and coronary artery disease (4-10). These investigations have produced conflicting results partially because of the differences in the assay systems used by the various investigators (11-12) and the possible effects of age on fibrinolytic activity (13-14). Also, fibrinolytic activity usually has been assayed using single blood samples obtained from subjects at rest. Such a procedure is an insensitive means of assessing the integrity of the blood fibrinolytic system, since abnormalities in the response of the system may be detectable only when the system is stressed (15). Although under ordinary circumstances intense exercise constitutes a desirable means of testing the capacity of the blood fibrinolytic system (15), this type of stress is impractical for subjects with coronary artery disease. However, the diurnal fluctuations of fibrinolytic activity normally occurring in man (16-17) and presumably representing a response to internal, physiological stimuli, also provide an index of the functional capacity of the fibrinolytic system. Therefore, to determine whether coronary artery disease correlates with impaired fibrinolysis, we studied the diurnal fluctuations of blood fibrinolytic activity in young normal subjects, in older normal subjects, in subjects with symptomatic coronary artery disease without lipid abnormalities, and in subjects with type IV hyperlipoproteinemia, a condition associated with the premature development of coronary artery disease (18-19).

Methods

GROUP 1

Group 1 included 19 young normal subjects (12 men and 7 women), 19-27 years old (mean 20 years). Physical examination and laboratory data including lipid profiles were normal.

GROUP 2

Group 2 included 24 older normal subjects (17 men and 7 women), 35-59 years old (mean 44 years). None
of these subjects had a history of angina pectoris, symptoms suggestive of peripheral vascular disease, overt diabetes mellitus, or electrocardiographic evidence of ischemic heart disease. Their lipid profiles were normal.

GROUP 3

Group 3 included 20 men with type IV hyperlipoproteinemia, 38–54 years old (mean 44 years). Thirteen of these subjects had angina pectoris with major coronary artery disease, i.e., narrowing of one or more coronary arteries by at least 50%, documented by arteriography. The remaining 7 subjects had no symptoms or electrocardiographic evidence of ischemic heart disease and did not undergo arteriography. Plasma triglyceride levels were above 250 mg/100 ml plasma (normal range in 50–59 year old subjects 10–190 mg/100 ml plasma), and a prominent pre-beta-lipoprotein band was observed by paper electrophoresis (20).

GROUP 4

Group 4 included 16 patients (13 men and 3 women), 41–64 years old (mean 52 years). All of these subjects had angina pectoris and major coronary artery disease documented by coronary arteriography, but no lipid abnormalities were detected.

No subject had engaged in any physical conditioning program for 1 month prior to study, although a few subjects in groups 3 and 4 may have been somewhat less active than the rest. All subjects received regular hospital diets for at least 3 days prior to the study; none had suffered a clinical myocardial infarction during the previous 3 months. Studies began at 8 AM after at least 8 hours of bed rest and a minimum of 12 hours of fasting. All subjects remained in bed throughout the study and ate regular hospital meals after the first, second, and fourth blood samples were drawn. They had no food between meals and were not allowed to smoke.

BLOOD ASSAYS

Total cholesterol (21), triglycerides (22), and lipoprotein paper electrophoresis (20) determinations were performed on plasma samples obtained the morning of the study. The procedures for measuring fibrinolytic activity, plasminogen, and fibrinogen have been described previously (17). The assays were performed on a random basis without any segregation by group. Blood samples for fibrinolytic activity were collected every 3 hours between 8 AM and 8 PM from an antecubital vein; the blood flowed freely from an 18-gauge needle into a chilled centrifuge tube containing 3.2% trisodium dihydrate (one part citrate solution, nine parts blood). The samples were placed in iced water and brought to the laboratory in an ice-cooled container. Samples of platelet-poor plasma, prepared in a refrigerated centrifuge and kept at -20°C, were used for the assays. Fibrinolytic activity of a plasma euglobulin fraction, determined by the fibrin plate method (23), appears to reflect a change in plasminogen activator activity (24-26). Fibrinolytic activity was expressed as the diameter product (mm²) of the lysed zones, and each value was the mean of triplicate determinations. Optimal precipitation of plasminogen activator in the euglobulin fraction occurred at pH 5.9 (27).

STATISTICAL METHODS

We assumed that aging would be associated with an impairment of the fibrinolytic response (28) and that the presence of type IV hyperlipoproteinemia or premature coronary artery disease would be associated with a further impairment. Since these hypotheses would be rejected if the diurnal increments of group 2 were equal to or greater than those of group 1 or if the increments of group 3 or 4 were equal to or greater than those of group 1 or 2, a single-tailed t-test was used for statistical comparisons. The statistical significance of the differences was evaluated by Student's t-test for paired or unpaired data and by chi-squared analysis. Data were expressed as means ± SE.

Results

GROUP 1

In each subject fibrinolytic activity increased during the day and attained peak levels at 5 PM or 8 PM (Fig. 1). The mean fibrinolytic activity was 69 ± 6 mm² at 8 AM; at peak activity it was 254 ± 14 mm². Fibrinolytic activity had increased at least 75 mm² at 5 PM in 18 of the 19 subjects and at least 100 mm² at 8 PM in all subjects relative to the lowest activity observed during the 12-hour period.
GROUP 2
These subjects did not show the same consistent diurnal increases in fibrinolytic activity that were observed in group 1 (Fig. 2). Only 13 of the 24 subjects achieved an increase of 75 mm² at 5 PM, and only 14 achieved an increase of 100 mm² at 8 PM. The mean activity at 8 AM was 71 ± 6 mm², and the mean peak activity was 202 ± 22 mm².

GROUP 3
The majority of these subjects showed little increase in fibrinolytic activity during the day (Fig. 3). Only 5 of the 20 subjects achieved an increase of 75 mm² at 5 PM and only 3 achieved an increase of 100 mm² at 8 PM. The mean activity at 8 AM was 90 ± 7 mm², and the mean peak activity was 137 ± 14 mm².

GROUP 4
The majority of these subjects also exhibited little diurnal change in fibrinolytic activity (Fig. 4). Only 3 of the 16 subjects achieved an increase of 75 mm² at 5 PM, and only 6 achieved an increase of 100 mm² at 8 PM. The mean activity at 8 AM was 85 ± 9 mm², and the mean peak activity was 164 ± 17 mm².

**FIGURE 2**
Diurnal variations in fibrinolytic activity in 24 older normal subjects (group 2). Open circles with horizontal lines indicate mean values at 8 AM and 8 PM.

**FIGURE 3**
Diurnal variations in fibrinolytic activity in 20 subjects with type IV hyperlipoproteinemia (group 3). Open circles with horizontal lines indicate mean values at 8 AM and 8 PM.

**FIGURE 4**
Diurnal variations in fibrinolytic activity in 18 patients with coronary artery disease but with normal lipid profiles (group 4). Open circles with horizontal lines indicate mean values at 8 AM and 8 PM.

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TWENTY-FOUR HOUR DIURNAL PATTERNS

Our previous investigations showed that fibrinolytic activity usually peaked at or before 8 PM (17). This observation was confirmed in the present study in four subjects with documented coronary artery disease. Three of these subjects had normal lipid levels and one had type IV hyperlipoproteinemia.

REPEAT DIURNAL STUDIES

In 12 subjects with coronary artery disease, 7 with normal lipid patterns and 5 with type IV hyperlipoproteinemia, 12-hour diurnal studies were repeated from 2 weeks to 13 months after the initial study. Paired data analysis revealed no significant difference between the peak activities of the two studies. Normal responses are defined as those showing increases not less than 75 mm$^2$ at 5 PM and 100 mm$^2$ at 8 PM; in both studies, 3 subjects exhibited a normal pattern and 8 subjects demonstrated impaired fibrinolysis at either 5 or 8 PM. Only 1 subject manifested a normal response in one study and an impaired fibrinolysis in the other.

INTERGROUP COMPARISONS

Since it is not known whether the peak diurnal fibrinolytic activity or the diurnal increment of fibrinolytic activity has the greater physiological significance or provides the better assessment of responsiveness, we used both values to compare fibrinolysis among the four groups.

Figure 5 shows that group 2 had a significantly lower mean peak fibrinolytic activity than did group 1 ($P<0.05$) and that group 3 had a significantly lower mean peak than did group 2 ($P<0.01$). Group 4 had a lower mean peak activity than did group 2, but the difference was not statistically significant.

To compare diurnal increments of fibrinolytic activity, each subject was classified according to whether he attained an increase of 75 mm$^2$ at 5 PM and 100 mm$^2$ at 8 PM above his lowest value. The percent of subjects exhibiting such increases is shown in Figure 6. At both times group 2 had significantly fewer subjects with the normal response than did group 1. Group 3 had fewer subjects with normal responses at both times, but the difference was significant only at 8 PM. Group 4 also exhibited a lower percent of subjects with
normal responses than did group 2, but the differences were not statistically significant.

Levels of Fibrinogen and Plasminogen

The mean plasma fibrinogen and plasminogen levels in each group at 8 AM on the first day of the study are shown in Table 1. Group 1 had a lower mean plasma fibrinogen level than did the other groups (P < 0.05), but all individual values were within the normal range. The mean plasma fibrinogen level in group 2 was not significantly different from that in group 3 or group 4. There was no significant difference between the mean plasminogen levels.

Discussion

The present investigation indicated that the diurnal increases of fibrinolytic activity normally occurring in young normal subjects diminished or disappeared in a significant number of older normal subjects. Moreover, the diurnal fibrinolytic responses of a significant number of subjects with type IV hyperlipoproteinemia were also reduced, and the reduction appeared to be greater than that which could be attributed to the effects of age alone.

The conflicting results of several other studies (13, 14, 29) in which the effects of aging on fibrinolysis were examined may have been due, in some instances, to differences in the assay systems used to measure the fibrinolytic activity. However, our results indicated that the failure to demonstrate a difference could have resulted from using one resting sample taken early in the day as the basis for comparison or from studying an abbreviated diurnal response that did not include peak fluctuations (14, 29). For example, if only 8 AM resting levels of fibrinolytic activity had been compared in the present study, it would have appeared that blood fibrinolytic activity was unaltered by the aging process (Table 1). Clear-cut differences were discerned only when peak diurnal fluctuations were considered.

Although it seems reasonable to attribute the poor responsiveness of the fibrinolytic system in a significant number of older normal subjects to some effect associated with aging, significant differences in plasma cholesterol and fibrinogen levels existed between the older and younger subjects (Table 1). However, in neither age group was there a significant correlation between plasma cholesterol and peak fibrinolytic activity (r = -0.20 for young normals, r = -0.01 for older normals). Also, although increased fibrinogen content in blood clots delays plasmin-induced clot lysis (30), the assay of the euglobulin fraction of the plasma on standardized bovine fibrin plates diminishes any effect of increased plasma fibrinogen levels. Therefore, the differences in the levels of plasma cholesterol or fibrinogen are probably not responsible for our findings. However, the increased plasma fibrinogen levels of the older normal subjects, the subjects with type IV hyperlipoproteinemia, and the subjects with coronary artery disease might impair fibrinolytic function in vivo and thereby delay, in a physiologically important way, the dissolution of intravascular fibrin deposits.

Using the euglobulin fraction of plasma to isolate plasminogen activator and assay fibrinolytic activity largely eliminated the contribution of inhibitors of the fibrinolytic system. A method described previously (17) showed no obviously elevated levels of inhibitors to tissue activator, urokinase, or plasmin in any subject, but we recognize that an optimal assay for inhibitors is not available. Thus, the euglobulin and other methods presently used to assay fibrinolytic activity may not provide a true indication of in vivo net fibrinolytic potential because of this less than optimal treatment of inhibitors.

Although many previous studies (4-6, 31-33) have attempted to relate elevated levels of blood lipids or

| TABLE 1 |
| Lipids, Fibrinogen, Plasminogen, and Diurnal Responses in Fibrinolytic Activity |

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/100 ml plasma)</th>
<th>Triglycerides (mg/100 ml plasma)</th>
<th>Fibrinogen (mg/100 ml plasma)</th>
<th>Plasminogen (% of pooled control)</th>
<th>Fibrinolytic activity (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>171 ± 7</td>
<td>73 ± 7</td>
<td>240 ± 7</td>
<td>87 ± 2</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>2</td>
<td>212 ± 7</td>
<td>73 ± 8</td>
<td>288 ± 12</td>
<td>89 ± 2</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>248 ± 9</td>
<td>482 ± 70</td>
<td>310 ± 15</td>
<td>83 ± 3</td>
<td>90 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>227 ± 10</td>
<td>114 ± 7</td>
<td>305 ± 11</td>
<td>84 ± 4</td>
<td>85 ± 9</td>
</tr>
</tbody>
</table>

All values are means ± se.
the existence of symptomatic atherosclerotic disease to defects in fibrinolysis, they have yielded conflicting results. The discrepancies may be related not only to different assay systems but also to inadequate protocol safeguards for eliminating the effects of aging and diurnal fluctuations on the interpretation of the fibrinolytic data. In addition, some investigations of the relation of coronary artery disease to blood fibrinolysis were performed immediately after an acute myocardial infarction (4-6) when the subjects were often receiving a variety of pharmacologic agents. Both the stress of the acute disease process (9) and the administration of various biologically active agents tend to seriously complicate the interpretation of data from such studies. Moreover, assaying only basal levels of fibrinolytic activity does not test the capacity of the fibrinolytic system to respond to stress. Our finding of a defective diurnal rise extends the results of a previous investigation (15) demonstrating that seven of eight subjects with type IV hyperlipoproteinemia had an impaired fibrinolytic response to exercise. Thus, these observations suggest that the accelerated development of coronary artery disease in subjects with type IV hyperlipoproteinemia may be related not only to the increased plasma levels of triglycerides but also to a coexisting defect in the responsiveness of the fibrinolytic system.

The majority of subjects with type IV hyperlipoproteinemia demonstrated both an impaired fibrinolytic response to exercise (15) and an impaired diurnal fibrinolytic increase. In contrast, although nearly half of the older normal subjects showed an impaired diurnal fibrinolytic increase, a previous study demonstrated that the fibrinolytic response to exercise was normal in six of seven older normal subjects (15). These findings indicate that a selective impairment of the fibrinolytic response may develop in man: the diurnal increases in fibrinolytic activity are altered adversely by aging, whereas the exercise-induced increases are not altered by age. These results also emphasize the importance of considering the influence of various stimuli that augment fibrinolytic activity when attempting to draw specific conclusions about fibrinolytic responsiveness and function in a particular group of subjects.

Therefore, our results suggest that a relation exists between impaired responsiveness of the fibrinolytic system and development of coronary artery disease. However, it should be emphasized that the physiological significance of the diurnal fibrinolytic response remains to be determined; the role of impaired fibrinolysis in the pathogenesis of atherosclerosis must presently be regarded as conjectural.

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