Increased levels of an endogenous inhibitor of tissue-plasminogen activator (t-PA) have been thought to relate to the genesis of acute myocardial ischemia. To examine the role of the rapid inhibitor of t-PA, plasma samples were analyzed from 75 patients with chest pain syndrome undergoing coronary angiography (mean age 57 years), 24 patients with clinically documented coronary artery disease (unstable angina, positive exercise stress test or previous history of myocardial infarction; mean age 58 years) and 15 young normal subjects (mean age 26 years). Plasma t-PA inhibitor levels were similar in age-matched patients regardless of the absence or presence (and degree) of coronary artery disease. Plasma t-PA inhibitor levels correlated significantly with age ($r = 0.46$, $p < 0.005$), suggesting an age-dependent decrease in fibrinolytic activity. Plasma t-PA inhibitor levels also correlated significantly with serum triglyceride levels ($r = 0.60$, $p < 0.001$), but not with coronary risk factors such as serum cholesterol, diabetes, hypertension, serum uric acid levels or body weight.

Association of high levels of inhibitor of t-PA with hypertriglyceridemia may be of importance in the development of coronary thrombosis, especially in elderly patients. Nonetheless, this study does not suggest a pathogenic role of t-PA inhibitor in coronary atherosclerosis.

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Methods

Subjects. Peripheral blood samples from 75 patients (74 men and 1 woman, aged 33 to 85 years) with a chest pain syndrome who were undergoing diagnostic coronary angiography were assayed for tissue plasminogen activator (t-PA) inhibitor levels. The extent of coronary atherosclerosis in these subjects was graded on a scale of 0 to 3 depending on the number of vessels with a luminal diameter reduction of greater than 50%.

Plasma samples from 24 outpatients (aged 34 to 72 years) with clinically documented coronary artery disease (previous history of myocardial infarction, positive exercise stress test or unstable angina) were also assayed for the inhibitor levels. Plasma samples from 5 pediatric subjects (aged 5 to 17 years) and from 10 healthy, normal laboratory personnel (aged 25 to 54 years) were also examined.
Measurement of the inhibitor of tissue-plasminogen activator (t-PA). Peripheral venous blood samples were collected from all subjects in trisodium citrate only between 8:00 and 9:00 A.M. because diurnal variation has been described in the plasma levels of the inhibitor (10). Blood was centrifuged at 4°C for 20 minutes at 1,500 g and plasma stored at −70°C. The plasma levels of the inhibitor were measured by an amidolytic assay using purified t-PA and a chromogenic substrate (3,10). In brief, t-PA is added to multiple dilutions of plasma, and residual t-PA activity is subsequently determined using the plasmin substrate p-Val-Leu-Lys-p-nitroaniline (S2251), a gift from Kabi-Vitrum AB, Stockholm. Plasmin inhibitors such as alpha2-antiplasmin and alpha2-macroglobulin are destroyed by acidification. One unit of the inhibitor is the amount inhibiting one unit of t-PA in this system.

Coronary risk factors. Serum levels of total cholesterol, triglycerides and uric acid were measured by standard enzymatic methods after a 12 hour fast in the 75 patients undergoing cardiac catheterization. The subjects were considered hypertensive if the supine diastolic blood pressure was greater than 90 mm Hg on at least two occasions. Smoking history, expressed as pack-years, was obtained by personal interview with the patients. Patients were considered to have diabetes mellitus if they were taking hypoglycemic agents or if they had been previously told they were diabetic and were treated with dietary control.

Statistical analysis. Group differences between patients with and without significant coronary atherosclerosis were tested for significance by the nonparametric Kruskal-Wallis one-way analysis of variance and the Mann-Whitney test. The relation between plasma levels of the t-PA inhibitor and coronary risk factors was examined by conventional linear regression analysis.

Results

Plasma tissue-plasminogen activator (t-PA) inhibitor levels. The data on the 75 patients with angiographic evidence of absence or presence (as well as extent) of coronary atherosclerosis are presented in Table 1. In these subgroups of patients with similar age but different extent of disease, t-PA inhibitor levels were not significantly different (p = NS). Serum levels of triglyceride, total cholesterol and uric acid as well as smoking pattern and prevalence of hypertension and diabetes mellitus were similar in these subgroups of patients. The t-PA inhibitor levels in patients with clinical evidence of coronary artery disease (3.02 ± 1.51 units/ml, mean ± SD) were also in the same range as in the patients undergoing coronary angiography (Fig. 1). However, compared with levels in healthy, normal laboratory personnel (mean 0.99 ± 0.64 units/ml) and pediatric subjects (mean 1.17 ± 0.95 units/ml), t-PA inhibitor levels in patients with clinically or angiographically documented coronary disease were significantly (p < 0.02) increased.

Plasma tissue-plasminogen activator (t-PA) inhibitor levels and coronary risk factors. The t-PA inhibitor levels correlated significantly with age (Fig. 2) (r = −0.46, p < 0.005), rising in older patients and were also correlated significantly with serum triglyceride levels (Fig. 3) (r = −

| Table 1. Angiographic Extent of Coronary Artery Disease, Tissue-Plasminogen Activator (t-PA) Inhibitor Levels and Coronary Artery Disease Risk Factors in 75 Patients Undergoing Coronary Angiography |
|---------------------------------|---|---|---|---|
| CAD Severity (no. of vessels diseased) |
| 0 | 1 | 2 | 3 |
| No. of patients | 14 | 11 | 21 | 29 |
| Age (yr) | 56 ± 12 | 56 ± 7 | 58 ± 8 | 61 ± 8 |
| Weight (kg) | 79.7 ± 16.2 | 87.3 ± 11.3 | 79.2 ± 10.4 | 79.7 ± 10.4 |
| Plasma t-PA inhibitor (units/ml) | 2.5 ± 1.4 | 2.2 ± 1.6 | 2.2 ± 1.7 | 2.2 ± 1.4 |
| Serum triglycerides (mg/dl) | 310 ± 196 | 231 ± 150 | 220 ± 254 | 192 ± 59 |
| Serum cholesterol (mg/dl) | 186 ± 54 | 200 ± 104 | 201 ± 49 | 175 ± 40 |
| Serum uric acid (mg/dl) | 5.8 ± 2.0 | 8.0 ± 2.2 | 5.9 ± 0.8 | 6.1 ± 2.5 |
| Smoking (pack-yr) | 39 ± 14 | 45 ± 31 | 42 ± 12 | 37 ± 16 |
| Hypertension (no. of patients) | 7 | 7 | 10 | 18 |
| Diabetes mellitus (no. of patients) | 2 | 2 | 4 | 8 |

Data expressed as mean values ± SD; CAD = coronary artery disease.
Figure 1. Tissue-plasminogen activator (t-PA) inhibitor levels in 10 young normal subjects (mean age 26 years), 75 patients with chest pain undergoing angiography (mean age 57 years) and 24 patients with clinical coronary artery disease (CAD) (mean age 58 years). Plasma t-PA inhibitor levels were significantly higher in the older patients than in the younger normal subjects.

![Graph showing plasma t-PA inhibitor levels](image)

0.60, p < 0.001). There was, however, no correlation between t-PA inhibitor levels and the serum concentrations of total cholesterol (Fig. 4), uric acid, patients' body weight, history of smoking, presence of hypertension or diabetes mellitus. The mean plasma value of the t-PA inhibitor was 2.57 ± 1.47 units/ml in normotensive subjects and 2.14 ± 1.44 units/ml in hypertensive subjects (p = NS). It was 2.32 ± 1.47 units/ml in patients without diabetes mellitus and 1.96 ± 1.41 units/ml in patients with diabetes mellitus (p = NS).

Discussion

**Age and t-PA inhibitor.** The results of our study demonstrate that patients with coronary artery disease have higher plasma levels of tissue-plasminogen activator (t-PA) inhibitor than do young normal, healthy subjects, and that the plasma levels of the inhibitor do not vary with the extent of the disease (7). Furthermore, we found no significant difference between age-matched patients with chest pain without significant coronary disease and others with signif-

![Graph showing positive correlation between age and plasma t-PA inhibitor levels](image)
significant disease relative to the plasma levels of t-PA inhibitor. These data strongly suggest that high levels of t-PA inhibitor are not primarily associated with coronary disease. Nevertheless, we found a significant correlation between the plasma levels of t-PA inhibitor and age of the subjects. Because the patients with coronary artery disease are generally older than those without disease, age is probably a contributory factor to the difference in t-PA inhibitor levels observed previously.

An influence of age on defective fibrinolysis as determined by the dilute blood clot lysis time has earlier been reported, but the results are conflicting. Chakrabarti et al. (9) initially described an increase in fibrinolytic activity with age in patients with coronary artery disease. However, these investigators (11) later found reduction in fibrinolytic activity after analysis of a large number of such patients. The clot lysis time used by these investigators is nonspecific and dependent on both plasminogen activators and various fibrinolysis inhibitors.

Serum triglycerides and tissue-plasminogen activator (t-PA) inhibitor. Another factor that may have contributed to the increased plasma levels of t-PA inhibitor in our pa-

Figure 3. Positive correlation between serum triglyceride levels and plasma t-PA inhibitor levels ($r = 0.60$, $p < 0.001$).

Figure 4. Absence of significant correlation between serum total cholesterol levels and plasma t-PA inhibitor levels.
patients undergoing coronary angiography is the significant prevalence of hypertriglyceridemia. The correlation between high levels of triglycerides and t-PA inhibitor found in the present investigation confirms similar observations made previously by us (10) and recently by Hamsten et al. (12). Although our results suggest that t-PA inhibitor is not of pathogenetic importance for coronary atherosclerosis, it is possible that hypertriglyceridemia may contribute to coronary thrombosis by inducing an increase in t-PA inhibitor levels, especially in elderly patients. That an increase in t-PA inhibitor may be secondary to the increase in triglycerides is favored by the demonstrated association of dietary regulation of triglycerides and increased fibrinolytic activity (13), which may be due to a reduction in t-PA inhibitor levels. At present, however, we cannot exclude a common factor, such as increased levels of catecholamines (12), which may cause elevation of both serum triglycerides and t-PA inhibitor levels, particularly in patients under stress. Nevertheless, coronary thrombosis may relate to reduced fibrinolytic activity, which has been found in patients with coronary artery disease who later died (14). Reduced fibrinolytic activity has also been associated with graft occlusion in patients undergoing aortocoronary bypass surgery (15).

Tissue-plasminogen activator (t-PA) inhibitor and fibrinolysis. Inhibitor of t-PA is probably produced by endothelial cells (16) as well as in platelets (17). Increased levels of this inhibitor were found in patients with the adult respiratory distress syndrome after trauma and sepsis (18), possibly related to endotoxin-induced release of t-PA inhibitor from the endothelial cells (5). A high level of t-PA inhibitor seems also to be responsible for the impaired fibrinolytic response to venous occlusion found in patients with deep venous thrombosis (19), related probably to rapid inactivation of the released t-PA by the inhibitor. The t-PA inhibitor rapidly forms a stable, inactive complex with the t-PA (20) and this mechanism may be of importance for delayed fibrin degradation and development of thrombosis. A protraction of whole blood and plasma clot lysis in patients with naturally occurring high levels of t-PA inhibitor which suggests a pathophysiologic significance of t-PA inhibitor in fibrinolysis and thrombolysis, was recently demonstrated (21). An in vivo effect of t-PA inhibitor on fibrin degradation and thrombolysis, however, has yet to be proved. Interestingly, an identical impaired response to fibrinolytic stimulus found in patients with thrombosis and high levels of the t-PA inhibitor (19) was demonstrated in patients with hypertriglyceridemia (22), further suggesting a connection between triglycerides, t-PA inhibitor, and thrombosis.

Normal values of tissue-plasminogen activator (t-PA) inhibitor. An international standard for t-PA inhibitor is not yet available, and the levels obtained in healthy, normal subjects differ among various laboratories. Mean values of 1.5 ± 0.7 (7), 0.46 ± 0.50 (8) and 0.7 ± 0.7 units/ml (23) have been reported. Plasma t-PA levels reported in patients with coronary artery disease range from 0.80 ± 1.19 (8) to 3.1 ± 1.2 units/ml (7). These differences can partly be explained by variations in age and triglyceride levels, and also by the diurnal change in the inhibitor levels (10). They could also be due to lack of uniformity in assays for measurement of the inhibitor; for example, it has been reported that the rate of inhibition of t-PA increases with the amount of t-PA added to plasma (24).

Conclusions. Hypertriglyceridemia, especially in elderly patients with coronary artery disease, is associated with increased plasma levels of t-PA inhibitor. This increase in the t-PA inhibitor levels might be of importance in the evolution of coronary thrombosis in these patients and could possibly be involved in the impaired response to t-PA treatment of coronary thrombosis in some patients. The fact that no difference in t-PA inhibitor levels was found between age-matched patients with and without coronary artery disease (and variable extent) speaks against, but does not exclude, an important role for t-PA in the development of coronary atherosclerosis.

References
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