Plasminogen activator levels are influenced by location and varicosity in greater saphenous vein

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Purpose: The plasminogen system, which includes tissue type plasminogen activator (tPA), urokinase type plasminogen activator (uPA), and their main inhibitor, plasminogen activator inhibitor type 1 (PAI-1), plays a major role in both fibrinolysis and tissue remodeling. This study compares the levels of tPA, uPA, and PAI-1 at the groin and ankle in normal and varicose greater saphenous vein (GSV).

Methods: GSV was collected from patients undergoing varicose vein (VV) removal and from normal vein (NV) from arterial bypass procedures. Portions of the GSV at the groin and the ankle were minced and placed in serum-free media for 48 hours. Assays of the supernatants were obtained for tPA, uPA, and PAI-1 protein by enzyme-linked immunosorbent assay. Cyclohexamide and actinomycin D were also added to the media of the VV tissue explant supernatants to inhibit protein and RNA synthesis, respectively.

Results: Levels of tPA were significantly higher at the groin (11 ± 2) than the ankle (5 ± 1) in the VV ($p < 0.005$), and this trend was also seen in the NV (groin 10 ± 2 and ankle 7 ± 3). Levels of uPA were significantly higher in the groin VV (14 ± 4.3) than in NV (3.0 ± 0.8, $p < 0.05$). This difference, although not statistically significant, applied to the ankle as well (VV 14.5 ± 6.3 and NV 5.3 ± 2.7). No significant difference was seen between NV and VV for PAI-1 (NV, groin 155 ± 73 and ankle 113 ± 53, VV, groin 161 ± 20 and ankle 142 ± 38) or tPA. Inhibitor studies revealed no significant difference among control, cyclohexamide, and actinomycin D supernatants for tPA, suggesting release of protein rather than active synthesis. In contrast, inhibitor supernatants were significantly lower for uPA and PAI-1 than control supernatants ($p < 0.05$), suggesting that uPA and PAI-1 were actively synthesized.

Conclusions: In the tissue explant supernatant model uPA and PAI-1 are actively synthesized, but tPA is not. Levels of PAI-1 were comparable in all four groups. Levels of uPA in the varicose GSV were higher than in NV, suggesting a role for uPA in the pathologic makeup of VV. Levels of tPA were higher at the groin versus the ankle position, potentially explaining the previously described increased fibrinolytic activity seen at the groin. (J Vasc Surg 1996;24:719-24.)

Varicose veins (VV) are present in 30% to 60% of adults and increase in incidence with age.[1] At least three current theories explain the pathogenesis of VV formation including valvular incompetence, multiple arteriovenous communications leading to increased

flow, and venous wall weakness.[2] Several investigators have evaluated the collagen content of VV to explore the theory of wall weakness. This evaluation has produced conflicting reports of decreased collagen content in VV by some authors,[3,4] whereas others have found increased amounts.[5,6] The plasminogen activation system is involved with tissue remodeling through its ability to degrade extracellular matrix components and therefore may play a role in VV formation.

Rose and Ahmed[7] examined the histologic characteristics of normal and VV and found increased fibrous tissue especially in the muscle layer, which caused separation and interruption of the muscle cell pattern and subintimal deposition of collagen in VV.

Transmission electron microscopy revealed increased fibrous tissue in VV separating the muscle fibers, and many of the collagen fibers lost their regular bundle formation. Some of the collagen appeared to have been ingested by the muscle cells, suggesting that these cells had taken on phagocytic properties and that many of the elastic fibers were scattered and broken. The authors believed that the altered tissue remodeling exhibited by fragmentation of the elastin and muscle cell separation may interfere with contraction of the veins and thereby contribute to wall weakness.

Genetic factors have also been implicated in the pathogenesis of venous wall weakness. Cornu-Thenard et al.[8] studied the families of patients with VV and found that the risk of having VV for the children was 90% when both parents were affected, 25% for male patients and 62% for female patients when one parent had this disease, and 20% when neither parent was affected. Further support for a genetic influence stems from the work of Maurel et al.,[6] who measured the levels of various types of collagen in normal veins, normal appearing segments of veins from patients with VV, and dilated segments of VV. They found that VV had a higher content of collagen than normal vein and that no significant differences were present in the collagen content of normal appearing segments and dilated areas of vein from patients with VV. This finding suggests that there may be a general predisposition to varicosity formation throughout the venous system of susceptible individuals and that mechanical or other factors may influence the actual location of the varicosities.

The fibrinolytic potential of the venous system is currently an area of research interest. Several investigators have studied the fibrinolytic potential of greater saphenous veins (GSV) and have found a significantly higher level of fibrinolytic activity in the groin area when compared with the ankle in VV.[9,10] These results were also observed in specimens obtained from legs amputated because of arterial insufficiency.[11] The methods used, however, measured overall fibrinolytic activity by comparing zones of lysis on fibrin agar plates rather than the level of each specific plasminogen activator.

There are two different plasminogen activators. Tissue type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA) convert the proenzyme plasminogen into its active form, plasmin. Plasmin is important in tissue remodeling and fibrinolysis. Plasminogen activator inhibitor type I (PAI-1) is a major inhibitor of both tPA and uPA. Plasmin plays a key role in the degradation of the extracellular matrix by directly digesting matrix proteins and by activating the proteolytic matrix metalloproteinases.[12] Breast and lung cancer, which can be thought of as unregulated forms of tissue remodeling, exhibit elevated levels of uPA.[13] The formation of varicose veins requires extensive tissue remodeling, and thus the kinetics of the plasminogen activators are of interest.

Another important function of plasmin is fibrinolysis. Plasmin degrades fibrin-rich clots after being activated by clot-bound tPA.[14] Elevated levels of plasma PAI-1 induce a hypercoaguable state by inhibiting the plasminogen activators, and this finding has been associated with thrombotic complications such as deep venous thrombosis[15] and myocardial infarction.[16]

To date, no quantitative data are available on the levels of tPA, uPA, and PAI-1 in normal veins (NV) and VV. With the method of tissue explant supernatants, the levels of the plasminogen activators and PAI-1 in NV and VV at the groin and ankle positions have been measured to allow speculation on their influence over tissue remodeling and fibrinolysis.

METHODS

The following studies were performed with the approval of the Institutional Review Board of Northwestern University, and all patients gave informed consent. Varicose GSV was obtained from patients undergoing vein stripping, and normal vein (NV) was acquired from operations with GSV bypass grafts for lower extremity arterial insufficiency. Samples from both the ankle and groin were obtained from all the patients with VV, whereas NV specimens were collected from one site only from each patient. VV were obtained with a plastic stripper to remove the GSV from the groin to the ankle in patients with documented saphenofemoral reflux and symptomatic varicosities in one or both legs. NV specimens were obtained from patients without any visible varicosities or history of venous disease.

Tissue explant supernatants. In the operating room, specimens were placed on ice in holding media (Dulbecco's Modified Eagle's Medium, Ham's F-12, 16% fetal bovine serum, penicillin/streptomycin, and amphotericin B) and were processed within 1 to 2 hours. Using sterile technique, the tissue was washed with phosphate-buffered saline solution to remove residual thrombus, minced into 1×1 mm squares, weighed, and placed into 4 ml of Roswell Park Memorial Institute media with penicillin/streptomycin per 1 gm of tissue. The specimens were incubated at 37° C with 5% CO_2 for 48 hours.

The media and tissue were removed from the culture flasks and centrifuged, and the supernatant was aliquoted and stored at −80° C. Protein and RNA syntheses were inhibited, respectively, by cyclohexamide (25 μg/ml) and actinomycin D (5 μg/ml) in the VV supernatants. The inhibitors were dissolved in alcohol and added to the Roswell Park Memorial Institute media at time zero and were in contact with the tissue for 48 hours. Control flasks contained tissue from the same patient placed in Roswell Park Memorial Institute media plus alcohol only. Normal specimens were not used in inhibitor experiments because of sample size limitations.

Quantification of tPA, uPA, and PAI-1. Total tPA, uPA, and PAI-1 antigens were quantitated with TintElize tPA, TintElize uPA, and TintElize PAI-1 (Biopool International, Ventura, Calif.), respectively. These enzyme-linked immunosorbent assays recognize all forms of tPA, uPA, and PAI-1. Manufacturer's instructions for nonplasma samples were followed, and interassay variability was approximately 5%.

Statistics. Unpaired and paired Student's t tests were performed, and results were reported as significant at the $p < 0.05$ level. Results are reported as the mean ± SEM.

RESULTS

Tissue explant supernatants. Assays of PAI-1, tPA, and uPA were obtained in 48-hour supernatants from both the ankle and groin positions of eight patients with VV, who had an average age of 48 ± 3 years. Normal GSV at the groin location was obtained from seven patients with an average age of 62 ± 4 years and at the ankle position from five patients whose average age was 73 ± 5 years. Results of these assays are reported in Table I. No significant differences were found in PAI-1 levels in any of the four groups. Levels of tPA were twofold higher at the groin position as compared with the ankle in the VV specimens ($p < 0.005$). In the normal specimens tPA levels were higher at the groin than the ankle, but the difference was not statistically significant. No significant differences were found between VV and NV tPA levels at either the groin or ankle positions.

In contrast to these findings, uPA levels at the groin were approximately fivefold higher in VV as compared with NV supernatants ($p < 0.05$). Although the differences were not significant, this trend was also observed at the ankle position between VV and NV samples. No significant differences were found in uPA levels when the locations of the samples were compared.

Inhibitor studies were performed on the eight

VV specimens at both the ankle and groin positions to determine whether the proteins were being actively synthesized in the tissue explant supernatant model. Cyclohexamide was used to inhibit protein synthesis, and actinomycin D was used to inhibit RNA synthesis. Results of these assays are reported in Table II. No significant differences were found in tPA levels in control versus treated supernatants, suggesting that tPA is released into the medium and is not actively synthesized in the tissue explant supernatant model. In contrast, both uPA and PAI-1 control groups were significantly higher than treated supernatants, suggesting that these proteins were actively synthesized in the tissue explant supernatant model. Furthermore no significant differences were found between the levels of uPA or PAI-1 in the actinomycin D versus the cyclohexamide supernatants, suggesting that the rate of protein synthesis of these two molecules was controlled at the level of transcription. Levels of uPA in control supernatants were approximately nine- to tenfold higher than in the treated supernatants, whereas PAI-1 control supernatants were five- to 14-fold higher than treated supernatants.

DISCUSSION

Previous studies demonstrated an increased fibrinolytic activity in normal[11] and varicose[9,10] GSV at the groin versus the ankle position but were unable to identify the responsible protease. Our findings suggest that tPA may be responsible for this previously described difference. There was approximately a twofold increase in tPA levels in groin VV compared with ankle GSV when paired samples from eight different patients were compared ($p < 0.005$). Normally, tPA is produced by endothelial cells, and the amount produced varies with the location of the endothelial cells.[17] Our findings indicate that the venous groin specimens contained more tPA than the ankle samples, possibly suggesting that groin endothelial cells store more tPA than those at the ankle. Normal GSV obtained from two different sets of patients at the groin and ankle positions followed this pattern as well, although the difference was not statistically significant, perhaps because of the small sample sizes. Individual variation in tPA levels was observed in all the groups, with a range of 5 to 15 ng/ml observed in the groin VV. The use of paired specimens in the VV samples decreased the differences introduced by individual variation and may explain why a statistically significant difference was seen in the VV groups but not the NV specimens. No significant differences were found in uPA or

Table I. Levels of tPA, uPA, and PAI-1 in normal and varicose GSV at the groin and ankle positions

	NV groin (n = 7)	NV ankle (n = 5)	VV groin (n = 8)	VV ankle (n = 8)
tPA (ng/ml)	10 ± 2	7 ± 3	11 ± 2*	5 ± 1*
uPA (ng/ml)	3.0 ± 0.8†	5.3 ± 2.7	14.3 ± 4.3†	14.5 ± 6.3
PAI-1 (ng/ml)	155 ± 73	113 ± 53	161 ± 20	142 ± 38

All values mean ± SEM.
*$p < 0.005$.
†$p < 0.05$.

Table II. Levels of tPA, uPA, and PAI-1 in control versus treated supernatant groups in groin and ankle VV

	Groin (n = 8)			Ankle (n = 8)		
	Control	Cyclohexamide	Actinomycin D	Control	Cyclohexamide	Actinomycin D
tPA (ng/ml)	11 ± 2	11 ± 2	11 ± 2	5 ± 0	5 ± 1	5 ± 0
uPA (ng/ml)	11.1 ± 2.2*	1.2 ± 0.2*	1.2 ± 0.2*	13.9 ± 4.6†	1.4 ± 0.3†	1.4 ± 0.3†
PAI-1 (ng/ml)	178 ± 24*	13 ± 1*	15 ± 2*	130 ± 35‡	24 ± 4‡	19 ± 5‡

All values mean ± SEM.
*$p < 0.005$.
†$p < 0.05$.
‡$p < 0.01$.

PAI-1 levels when the groin and ankle positions were compared in these specimens.

Comparisons between normal and varicose specimens revealed a significantly higher level of uPA in groin VV versus NV ($p > 0.05$). This trend was also observed at the ankle position; however, the difference was not significant, perhaps because of the small sample size of five in the ankle NV group. Overall, levels of uPA were approximately three- to fivefold higher in VV than NV, whereas no significant differences were observed in tPA and PAI-1 levels. This finding suggests that uPA may be involved in the pathogenesis of VV formation, possibly through its ability to activate plasmin. Both uPA and plasmin are the principal proteases that activate the latent matrix metalloproteinases, including collagenase and elastase.[12] Thus, an increased uPA level may lead to enhanced breakdown of collagen and elastin. On histologic evaluation VV exhibit fragmented elastin and increased placement in the media of both structurally normal and abnormal collagen.[7] Activities of various enzymes in normal and varicose veins have previously been studied. Niebes and Laszt[18] evaluated enzymes involved in mucopolysaccharide metabolism and found increased activities of hyaluronidase, arylsulfatase, and glucosaminidase in VV compared with those in NV tissue. Urbanova and Prerovsky[19] studied hydrolytic enzymes and showed elevations in acid phosphatase and lactate dehydrogenase in VV. In contrast, Gandhi et al.[5] found no significant differences between varicose and normal vein gelatinolytic, caseinolytic, or elastolytic activities but found in-creased collagen and decreased elastin content in VV. These findings are consistent with alterations in tissue remodeling. It is more difficult, however, to explain the observations of fragmented elastin[7] and decreased elastin content in VV but no difference in elastolytic activity.[5] Perhaps elastin is destroyed by mechanisms other than an increased elastase activity, or the elastase activity is only transiently elevated and then returns to baseline in established varicosities.

Another feature of this disease that has generated interest is the observation that the biochemical profiles of varicosities and "normal appearing" segments of vein from patients with VV are similar. Maurel et al.[6] and Gandhi et al.[5] both found elevations of collagen and decreased levels of elastin[5] in varicosities and in normal segments of vein from patients with VV compared with that from patients with normal veins. Lowell et al.[20] studied varicose tributaries and non-varicose greater saphenous vein from the same patients and found that maximal contractions in response to potassium chloride, norepinephrine, and endothelin were reduced in both varicose and non-varicose GSV compared with control segments of vein. They also found attenuation of relaxation in the two vein groups from patients with VV and decreased protein content and increased endothelin levels compared with those in normal vein. Consistent with these findings is the work of Blochl-Daum et al.,[21] who studied the responsiveness of superficial hand veins to local infusions of noradrenaline in patients with primary varicose veins and healthy volunteers. They found that patients with VV required signifi-

cantly higher doses of noradrenaline for half-maximal venoconstriction than the dose required by a control group. Furthermore, the noradrenaline responsiveness in varicose veins was not significantly different from hand vein responsiveness in the same patient. These observations are consistent with the theory that there may be a general predisposition to varicosity formation throughout the venous system of susceptible individuals and that mechanical or other factors may influence the actual location of the varicosities. In this study most of the GSV removed from patients with VV were normal on gross evaluation. The groin area was more likely to be dilated than was the ankle location, and yet the uPA levels were similar in both of these areas but were elevated when compared with veins from normal individuals. Additional research in this area may further delineate the mechanisms of varicosity formation.

Of interest is the protein and RNA synthesis inhibition studies performed on the VV specimens. These results showed that uPA and PAI-1 were actively synthesized in the tissue explant supernatant model but that tPA was not. No significant differences were found in the levels of uPA and PAI-1 in the actinomycin D versus the cyclohexamide supernatants, suggesting that the rate of synthesis of these two molecules is controlled at the level of transcription. In contrast, similar inhibition experiments with tissue explant supernatants with normal and aneurysmal aorta revealed that PAI-1[22] was actively synthesized in this model but that tPA and uPA were not.[23] We can only speculate on why uPA is actively synthesized in the VV but not the aortic supernatants and whether uPA would be actively synthesized in NV supernatants. Further research is needed to elucidate these biochemical differences between the arterial and venous specimens.

A potentially confounding factor in this study is the differences in the ages of the VV group (48 ± 3 years) compared with the NV group (groin, 62 ± 4 and ankle, 73 ± 5 years). Most of the previous work dealing with levels of the components of the plasminogen system and age have been performed on serum samples with conflicting results. For PAI-1 some authors have reported an age-related increase in serum PAI-1 levels,[24-27] whereas others have found this age-related increase only in male[28] or female patients,[29] and, finally, some researchers report no association with serum PAI-1 levels and age.[30-32] Conflicting data for serum levels of tPA also exist, with most of the authors showing an age-related increase,[25-27,32-34] whereas another report reveals an age-related decrease.[27] A literature search failed to find any data regarding serum uPA levels and age. This report studied levels of various plasminogen system components in the venous wall, and no age-related differences were noted; however, the number of samples was small. We are not aware of any previous studies comparing venous wall levels of tPA, uPA, and PAI-1 and age. Other researchers have studied tissue levels of the plasminogen system and found no correlation with age of tPA levels in breast cancer[35] or PAI-1 levels in pulmonary adenocarcinoma[36] and brain tumors.[37] Increased levels of uPA, however, were associated with increased age in pulmonary adenocarcinoma.[36]

Another potentially confounding factor in this study relates to the myriad of associations of the different plasminogen system components with various disease states and lifestyles. For example, decreased serum PAI-1 levels have been found in people who consume a diet high in fruits and vegetables[31] and exercise,[38] whereas elevated levels of PAI-1 have been associated with familial venous thrombosis[15] and myocardial infarction.[16] Controlling for all these factors would be extremely difficult; therefore we cannot rule out the possibility that the observed associations in this study may be due to other factors.

Finally, antigen levels of tPA, uPA, and PAI-1 were measured in the tissue explant supernatant studies rather than the activity of these proteins caused by the instability of these molecules in the tissue culture media. PAI-1 is thought to be secreted in an active unstable form with a half-life of 75 minutes at 37° C.[39] Both uPA and tPA are secreted in a proenzyme form that is activated by plasmin cleavage. Active uPA and tPA in tissue culture media are rapidly inhibited by binding to the more abundant PAI-1[40]; therefore, there would be essentially no active components of the plasminogen system at the 48-hour time point chosen in this study.

To summarize, in the tissue explant supernatant model uPA and PAI-1 are actively synthesized, but tPA is not. Levels of PAI-1 were comparable between normal and varicose GSV and between the ankle and groin positions. Levels of uPA in the varicose GSV were significantly higher than in NV at both the groin and ankle positions, suggesting a role for uPA in the pathogenesis of VV. Levels of tPA were higher at the groin versus the ankle position in VV and tended to go in that direction with NV, potentially explaining the previously described increased fibrinolytic activity seen at the groin compared with the ankle level.

REFERENCES

1. Goldman MP, Weiss RA, Bergan JJ. Diagnosis and treatment of varicose veins: a review. Dermatology 1994;31:393-413.

2. Clarke GH, Vasdekis SN, Hobbs JT, Nicolaides AN. Venous wall function in the pathogenesis of varicose veins. Surgery 1992;111:402-8.

3. Andreotti L, Cammelli D. Connective tissue in varicose veins. Angiology 1979;30:789-805.

4. Psaila JV, Melhuish J. Viscoelastic properties and collagen content of the long saphenous vein in normal and varicose veins. Br J Surg 1989;76:37-40.

5. Gandhi RH, Irizarry E, Nackman GB, Halpern VJ, Mulcare RJ, Tilson MD. Analysis of the connective tissue matrix and proteolytic activity of primary varicose veins. J Vasc Surg 1993;18:814-20.

6. Maurel E, Azema C, Deloly J, Bouissou H. Collagen of the normal and the varicose human saphenous vein: a biochemical study. Clin Chim Acta 1990;193:27-38.

7. Rose SS, Ahmed A. Some thoughts of the aetiology of varicose veins. J Cardiovasc Surg 1986;27:534-43.

8. Cornu-Thenard A, Boivin P, Baud JM, De Vincenzi I, Carpentier PH. Importance of the familial factor in varicose disease: clinical study of 134 families. J Dermatol Surg Oncol 1994;20:318-26.

9. De Cossart L, Marcuson RW. Vascular plasminogen activator and deep vein thrombosis. Br J Surg 1983;70:369-70.

10. Wolfe JHN, Morland M, Browse NL. The fibrinolytic activity of varicose veins. Br J Surg 1979;66:185-7.

11. Ljungner H, Bergqvist D, Isacson S, Nilsson IM. Comparison between the plasminogen activator activity in the walls of superficial, muscle, and deep veins. Thromb Res 1981;22:295-302.

12. Murphy G, Atkinson S, Ward R, Gavrilovic J, Reynolds JJ. The role of plasminogen activators in the regulation of connective tissue metalloproteinases. Ann NY Acad Sci 1992;667:1-12.

13. Sappino A, Busso N, Belin D, Vassalli J. Increase of urokinase-type plasminogen activator gene expression in human lung and breast carcinomas. Cancer Res 1987;47:4043-6.

14. Saksela O, Rifkin DB. Cell-associated plasminogen activation: regulation and physiological functions. Ann Rev Cell Biol 1988;4:93-126.

15. Jorgensen M, Bonnevie-Nielsen V. Increased concentration of the fast-acting plasminogen activator inhibitor in plasma associated with familial venous thrombosis. Br J Haematol 1987;65:175-80.

16. Hamsten A. Wiman B, De Faire U, Blomback M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. N Engl J Med 1985;313:1557-63.

17. Levin EG, Loskutoff DJ. Comparative studies of the fibrinolytic activity of cultured vascular cells. Thromb Res 1979;15:869-78.

18. Niebes P, Laszt L. Research on the activity of certain enzymes in the metabolism of mucopolysaccharides in the normal and varicose human saphenous vein. Angiologica 1971;8:7-16.

19. Urbanova D, Prerovsky I. Enzymes in the wall of normal and varicose veins: histochemical study. Angiologica 1972;9:53-61.

20. Lowell RC, Gloviczki P, Miller VM. In vitro evaluation of endothelial and smooth muscle function of primary varicose veins. J Vasc Surg 1992;16:679-86.

21. Blochl-Daum B, Schuller-Petrovic S, Woltz M, Korn A, Bohler K, Eichler H. Primary defect in α-adrenergic responsiveness in patients with varicose veins. Clin Pharmacol Ther 1991;49-52.

22. Shireman PK, McCarthy WJ, Pearce WH, et al. Elevated levels of plasminogen activator inhibitor type 1 in atherosclerotic aorta. J Vasc Surg 1996;23:810-7.

23. Shireman PK, McCarthy WJ, Pearce WH, Kwaan HC. El-

evated levels of plasminogen activators in diseased aorta. Surg Forum 1995;46:362-4.

24. Mehta J, Mehta P, Lawson D, Saldeen T. Plasma tissue plasminogen activator inhibitor levels in coronary artery disease: correlation with age and serum triglyceride concentrations. J Am Coll Cardiol 1987;9:263-8.

25. Hashimoto Y, Kobayashi A, Yamazaki N, Sugawara Y, Takada Y, Takada A. Relationship between age and plasma tPA, PA-inhibitor, and PA activity. Thromb Res 1987;46:625-33.

26. Aillaud MF, Pignol F, Alessi MC, et al. Increase in plasma concentration of plasminogen activator inhibitor, fibrinogen, von Willebrand factor, factor VIII:C, and in erythrocyte sedimentation rate with age. Thromb Haemostasis 1986;55:330-2.

27. Krishnamurti C, Tang DB, Barr CF, Alving BM. Plasminogen activator and plasminogen activator inhibitor activities in a reference population. Am J Clin Pathol 1988;89:747-52.

28. Kario K, Matsuo T. Light-related hemostatic abnormalities in the elderly: imbalance between coagulation and fibrinolysis. Atherosclerosis 1993;103:131-8.

29. Sundell IB, Nilsson TK, Hellsten G, Hallmans G. Fibrinolytic values are related in age, sex, blood pressure and body build measurements: a cross-sectional study in Norsjo, Sweden. J Clin Epidemiol 1989;42:719-23.

30. Stegnar M, Keber D, Pentek M, Vene N, Kruft C. Age and sex differences in resting and postocclusion values of tissue plasminogen activator in a healthy population. Fibrinolysis 1988;2:121-2.

31. Nilsson TK, Sundell IB, Hellsten G, Hallman G. Reduced plasminogen activator inhibitor activity in high consumers of fruits, vegetables and root vegetables. J Int Med 1990;227:267-71.

32. Eliasson M, Evrin PE, Lundblad D. Fibrinogen and fibrinolytic variables in relation to anthropometry, lipids and blood pressure. The Northern Sweden Monica Study. J Clin Epidemiol 1994;47:513-24.

33. Margalione M, Di Minno G, Grandone E, et al. Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke. Arterioscler Thromb 1994;14:1741-5.

34. Ranby M, Bergsdorf N, Nilsson T, Mellbring G, Winblad B, Bucht G. Age dependence of tissue plasminogen activator concentration in plasma, as studied by an improved enzyme-linked immunosorbent assay. Clin Chem 1986;32:2160-5.

35. Yamashita J, Ogawa M, Inada K, et al. Breast cancer prognosis is poor when total plasminogen activity is low. 1993;67:374-8.

36. Pedersen H, Grondahl-Hansen J, Francis D, et al. Urokinase and plasminogen activator inhibitor type I in pulmonary adenocarcinoma. Cancer Res 1994;54:120-3.

37. Sawaya R, Yamamoto M, Ramo OJ, Shi ML, Rayford A, Rao JS. Plasminogen activator inhibitor-1 in brain tumors: relation to malignancy and necrosis. Neurosurg 1995;36:375-80.

38. Szymanski LM, Pate RR. Effects of exercise intensity, duration, and time of day on fibrinolytic activity in physically active men. Med Sci Sports Exerc 1994;26:1102-8.

39. Declerck PJ, De Mol M, Alessi MC, et al. Purification and characterization of a plasminogen activator inhibitor 1 binding protein from human plasma. J Biol Chem 1988;263:15454-61.

40. Loskutoff DJ, Van Aken BE, Seiffert D. Abnormalities in the fibrinolytic system of the vascular wall associated with atherosclerosis. Ann NY Acad Sci 1995;748:177-83.