

Fibrinolytic Response to Venous Occlusion Compared to Physical Stress Test in Young Patients with Coronary Artery Disease

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Key words

Coronary artery disease, fibrinolytic system, venous occlusion, physical stress test

Summary

Introduction: Venous occlusion (VO) and exercise stress (ES) are stimulators of the fibrinolytic system. Aim of this study was to answer which of both stimulation tests is more useful in patients with symptom-limited coronary artery disease (CAD) to evaluate possible defects in the fibrinolytic system.

Methods and results: We investigated 20 patients (M/F = 15/5; mean age = 36.7 years) with angiographically proven CAD for their plasma levels of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-type-1 (PAI-1) at basal conditions as well as after VO and at maximal ES (standardised bicycle stress test) and compared the data to those obtained from 12 sex- and age-matched healthy controls (M/F = 9/3; mean age = 40.4 years). At basal conditions mean t-PA activity and t-PA antigen plasma levels were within the normal range and comparable between the two study groups. After both VO and maximal ES, mean t-PA activity and t-PA antigen levels increased significantly more in the control group as compared to the CAD group. Mean PAI-1 activity plasma levels were significantly higher in the CAD group at basal conditions before VO (patients 7.0 ± 3.1 ; controls 3.9 ± 3.9 ; IU/ml; $p = 0.025$) as well as before ES (patients 8.1 ± 3.5 ; controls 4.3 ± 3.8 ; IU/ml; $p = 0.009$). PAI-1 activity plasma levels showed a significant decrease for patients and controls only after VO, while PAI-1 activity was not significantly decreased in both study groups at maximal ES.

Discussion: The significantly higher increase in mean plasma levels of t-PA activity and t-PA antigen after VO compared to ES in both groups might be explained by the fact that CAD induced symptoms in the patients during ES thus permitting only 80% of their age, sex, and body mass index related optimal work load.

Conclusion: VO and ES are applicable triggers of the endogenous fibrinolytic system in healthy subjects and patients who are not limited in their physical exercise. Standardised VO appears to be superior to ES as stimulation test of the endogenous fibrinolytic system in patients with symptomatic CAD.

Introduction

The fibrinolytic system of the vessel wall plays an important role in the prevention of local thrombus formation and initiation of endogenous thrombolysis. A defective fibrinolytic system is thought to support thrombus formation and can be explained by two main mechanisms: a reduced release of tissue-type plasminogen activator (t-PA) from diseased endothelial cells after a thrombotic stimulus and an increase of basal plasma levels of plasminogen activator inhibitor type-1 (PAI-1) (1). A release of t-PA from the vessel wall can be induced by a variety of stimuli, e.g. by venous occlusion (VO) of the forearm (2-11), by systemic administration of desmopressin (DDAVP) (2, 4), isoproterenol, or phenylephrine (12), or by physical exercise (8, 10, 13-17). An impaired release of tissue-type plasminogen activator (t-PA) after VO has been described mainly in patients with venous thrombosis (18-22) but only few data exist about the role of a diminished fibrinolytic response after VO (23-25) or after physical activity (26, 27) in atherothrombotic diseases, i.e. coronary artery disease (CAD) or cerebrovascular disease. Both Speiser (23) and Estelles (26) performed venous occlusion tests as well as exercise stress tests (EST) in their patients. However, these stimulation tests were performed in different populations and in different ways concerning duration and performance of test. Interpretation of the results is therefore sometimes difficult.

In this study we wanted to investigate which of both, VO or ES is the most applicable and preferable stimulation test in young patients with CAD to assess their fibrinolytic response.

Patients and Methods

Patients: We investigated 20 consecutive patients below 45 years with angiographically proven but clinically stable CAD (28) and compared the data obtained to a group of 12 age- and sex-matched individuals without evidence of CAD. Demographic data of patients and controls are given in Table 1.

Blood sampling: To minimise diurnal alterations of the fibrinolytic parameters, all blood collections were performed after a 12 h fast between 8.00 a.m. and 10.00 a.m. (29-35). First blood was drawn from an antecubital vein without or with only minimal VO at resting conditions. Then a standardised VO of 15 min duration was performed on the contralateral forearm (3, 25) followed by a second blood collection from the occluded extremity. Between 6 and 8 weeks after VO, plasma samples were collected again in all CAD patients and controls at rest and after a standardised bicycle stress test, with a 25 Watt increase of work load every 2 min, at the time point of maximal tolerable workload. In general, blood was drawn directly into plastic tubes coated with EDTA (final concentration of 5×10^2 M) and subjected to immediate centrifugation (4° C; 3000; 15 min) to separate cellular material from plasma. Aliquots of the individual plasma samples were then stored at -70° C until use.

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Determination of fibrinolytic parameters: t-PA antigen (measuring both t-PA and PAI-1 complexes, variation coefficient: intraassay 5% – interassay 10%) and t-PA activity plasma levels were determined by means of a combined assay system [Technoclone, Vienna (36)]. PAI-1 functional activity was determined using a modified functional titration assay (variation coefficient: ,10%) (37). Plasma concentrations of the fibrinolytic parameters after VO or EST were corrected for hemoconcentration by use of a correction factor as described: $K = (1 - \text{Hematocrit after}) / (1 - \text{Hematocrit before})$ (22, 38, 39).

Determination of routine parameters and cardiovascular risk factors: Hematocrit and platelet count were determined using laboratory routine assays. Plasma levels of cholesterol and triglycerides were measured using commercially available test kits and a multianalyzer system (Hitachi 705, Boehringer Mannheim, Germany).

Statistical analysis: If not stated otherwise, results are expressed as mean values and standard deviation ($\bar{x} \pm \text{SD}$). Between group comparison of continuous data was done with the Mann-Whitney U-test, within group comparison was done with the Wilcoxon test for matched pairs. Categories were compared with the Chi-square test, or Fisher's exact test if appropriate. A two-tailed p value of <0.05 was regarded as statistically significant. All calculations were done using a computer program (SPSS for Windows, version 6.0)

Results

As can be seen from Table 1 80% of patients had a history of myocardial infarction and smoked more frequently. Other cardiovascular risk factors, such as hypertension and hyperlipidemia were equally distributed between patients and controls; none of the study participants had diabetes mellitus.

As presented in Table 2, there was no difference between patients and controls with respect to hematocrit, platelet count or plasma levels of total cholesterol. However, triglyceride levels were significantly increased in patients with CAD.

Mean t-PA antigen and activity levels at basal conditions were not statistically different between the study groups (Table 3). Venous occlusion was in both groups followed by a significant increase of mean t-PA antigen and t-PA activity plasma levels, whereby t-PA antigen and t-PA activity increased to a significantly higher extent in the control group (Table 3). The mean increase of t-PA antigen levels in CAD patients accounted for only 60% of that of the control group ($p = 0.027$). Consistently, increase of t-PA activity was almost 3-times higher in controls as compared to patients (13.8 IU/ml vs. 5 IU/ml; $p = 0.016$).

Basal PAI-1 activity plasma levels were significantly higher in CAD patients compared to controls, but showed a similar decrease ($\Delta\text{PAI-1}$: patients: -4.8 U/ml; controls: -3.6 U/ml; $p = 0.15$) after VO for both study groups.

ES was performed between 6 and 8 weeks after VO. There were no significant differences in basal fibrinolytic parameters during this time interval for patients and controls (see Tables 3 and 4).

The maximal tolerable workload of patients with CAD was significantly lower compared to controls (144 vs. 179 Watt; $p < 0.05$). Accordingly, the percentage of maximal tolerable physical exercise as compared to age-, sex-, and body-mass-related normal individuals was only $76 \pm 18\%$ in patients but $107 \pm 35\%$ in controls (Table 4).

ES led to significant increase in t-PA activity levels in both study groups (Table 4) while t-PA antigen levels were slightly but not significantly increased. At peak exercise t-PA activity increased to an about 9-fold higher extent in controls compared to patients. At maximal exercise, PAI-1 activity levels decreased in a comparable way in both groups ($\Delta\text{PAI-1}$ activity: patients: -2.9 ; controls: -2.4 ; IU/ml; $p = \text{n.s.}$).

Table 1 Demographic data of patients and controls. CAD denotes coronary artery disease; VD denotes vessel disease; n.s. denotes not significant. Extent of disease is defined as number of main coronary arteries with at least one narrowing of $>70\%$ of the original vessel diameter

	PATIENTS	CONTROLS	p-value
n	20	12	
Male/Female	15/5	9/3	n.s.
Previous MI	16	-	
Age (years)			
- mean \pm SD	36.7 \pm 5.6	40.4 \pm 9.7	n.s.
- range	21-44	25-56	
Extent of CAD *			
- 0 VD	3		
- 1 VD	14	-	
- 2 VD	3	-	
- ≥ 3 VD	0	-	
Risk Factors (%)			
- smoking	85	17	<0.01
- hypertension	10	8	n.s.
- hyperlipidemia	60	50	n.s.
- diabetes	0	0	n.s.

Table 2 Blood lipids, hematocrit and platelet count in patients and controls. Data are given as median (and interquartile range)

	PATIENTS	CONTROLS	p-value
Cholesterol (mg/dl)	220 (IQR 195-243)	222 (IQR 162-246)	n.s.
Triglycerides (mg/dl)	163 (IQR 133-243)	135 (IQR 68-170)	0.045
Hematocrit (%)	43 (IQR 41-44)	42 (IQR 41-45)	n.s.
Platelets (x1000/mm³)	266 (IQR 237-330)	243 (IQR 226-247)	n.s.

Table 3 Hematocrit and fibrinolytic parameters in patients and controls before and after venous occlusion. VO denotes venous occlusion; n.s. denotes not significant; p-value^a comparison of data obtained between patients and controls; p-value^b comparison of data obtained before and after VO within the respective study groups

	PATIENTS	CONTROLS	p-value ^a
Hematocrit			
(% increase after VO)	11.1 \pm 1.6	11.3 \pm 2.3	n.s.
t-PA act (IU/ml)			
- before VO	0.08 \pm 0.34	0.08 \pm 0.30	n.s.
- after VO	5.03 \pm 4.35	13.9 \pm 11.65	0.015
p-value ^b	<0.001	<0.001	
Δt-PA act	5.0 \pm 4.3	13.8 \pm 11.7	0.016
t-PA ag (ng/ml)			
- before VO	4.9 \pm 2.6	4.3 \pm 2.1	n.s.
- after VO	18.1 \pm 11.9	24.6 \pm 12.1	0.045
p-value ^b	<0.01	<0.01	
Δt-PA ag	13.2 \pm 10.9	20.2 \pm 11.6	0.027
PAI-1 act (IU/ml)			
- before VO	7.0 \pm 3.1	3.9 \pm 3.9	0.025
- after VO	2.1 \pm 4.5	0.4 \pm 1.3	n.s.
p-value ^b	<0.01	<0.001	
ΔPAI-1 act	-4.8 \pm 5.4	-3.6 \pm 3.1	n.s.

In Figure 1 individual alterations of t-PA activity levels after VO (upper panel) or EST (lower panel) are given. As can be seen, VO stimulated the release of t-PA activity to a higher extent in both CAD patients and controls compared to ES. Similar results with less strik-

Table 4 Maximal tolerable workload, hematocrit and fibrinolytic parameters in patients and controls before and after exercise stress test. EST denotes exercise stress test; n.s. denotes not significant; p-value^a comparison of data obtained between patients and controls, p-value^b comparison of data obtained before and after EST within the respective study groups

	PATIENTS	CONTROLS	p-value ^a
Maximal workload during EST (Watt)	144.3±11.1	179.5±14.8	<0.05
Hematocrit (% increase due to EST)	4.1±1.3	2.8±1.5	n.s.
t-PA act (IU/ml)			
- before EST	0.0±0.0	0.08±0.3	n.s.
- after EST	0.6±1.0	5.7±5.4	0.003
p-value ^b	0.02	0.001	
Δt-PA act	0.6±1.0	5.6±5.4	0.003
t-PA ag (ng/ml)			
- before EST	5.3±2.9	4.3±2.1	n.s.
- after EST	8.6±6.0	8.6±4.7	n.s.
p-value ^b	n.s.	n.s.	
Δt-PA ag	3.2±4.0	4.2±3.6	n.s.
PAI-1 act (IU/ml)			
- before EST	8.1±3.5	4.3±3.8	0.010
- after EST	5.1±3.9	1.9±3.4	0.024
p-value ^b	n.s.	n.s.	
ΔPAI-1 act	-2.9±4.0	-2.4±5.3	n.s.

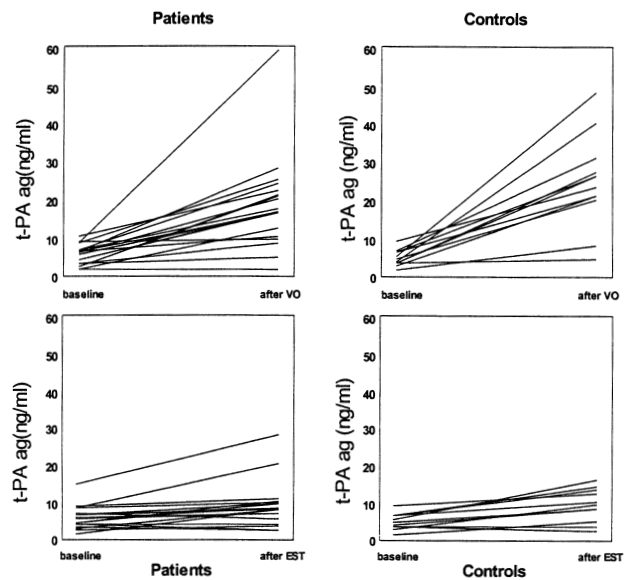


Fig. 2 Individual t-PA activity plasma levels before and after VO (upper panel) and before and at maximal tolerable workload (lower panel) in patients (left) and controls (right). VO denotes venous occlusion; EST denotes exercise stress test

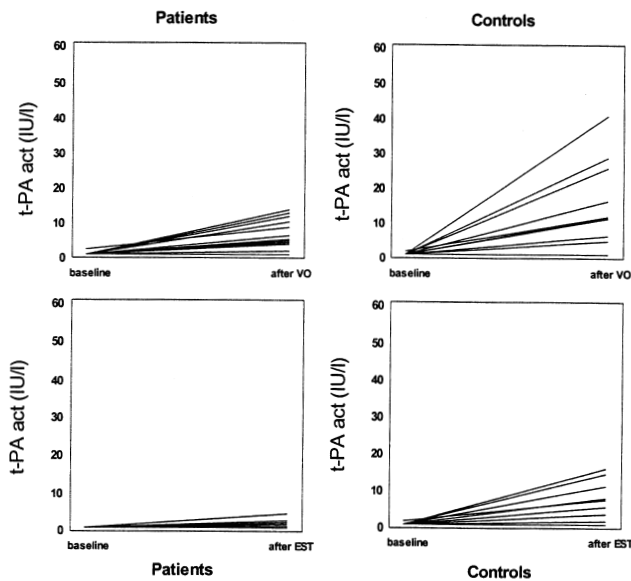


Fig. 1 Individual t-PA antigen plasma levels before and after VO (upper panel) and before and at maximal tolerable workload (lower panel) in patients (left) and controls (right). VO denotes venous occlusion; EST denotes exercise stress test

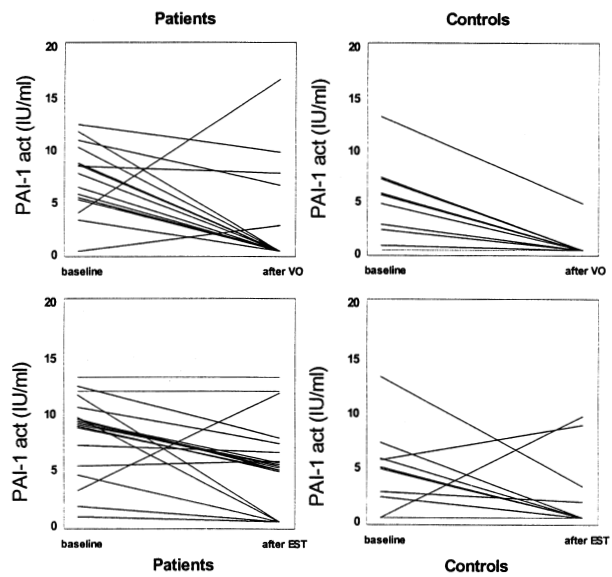


Fig. 3 Individual PAI 1 activity plasma levels before and after VO (upper panel) and before and at maximal tolerable workload (lower panel) in patients (left) and controls (right). VO denotes venous occlusion; EST denotes exercise stress test

ing differences could be shown for t-PA antigen (Fig. 2). PAI-1 activity decreased in most individuals of the two study groups (Fig. 3) similarly.

The fibrinolytic response was enhanced by venous occlusion when compared to exercised test in both patients and controls (<t-PA act 8.3 ± 8.9 IU/ml vs 2.5 ± 4.1, p < 0.001; <t-PA ag 15.8 ± 11.5 ng/ml vs 3.6 ± 3.8, p < 0.001; <PAI-1 act 4.4 ± 4.6 IU/ml vs 2.7 ± 4.6, p = 0.04).

Discussion

Up to now, the influence of VO or ES on fibrinolytic parameters has been investigated mostly in healthy individuals and data in patients with atherothrombotic disease, particularly in symptom-limited CAD patients, are rare. Standardised and reproducible stimulation test of the fibrinolytic system could, however, be helpful to identify pa-

tients at risk for clinical manifestations of atherothrombotic diseases (40).

VO is most efficient when the cuff is maintained inflated at mid pressure between systolic and diastolic for at least 15–20 min (3, 25). Shorter exposure to VO has been shown to produce a less strong and therefore often insignificant t-PA release in healthy subjects and is furthermore less reproducible (41, 42). From numerous experiences it seems to be evident that an increase in hematocrit of >10% of the baseline value is indicative for a correctly performed VO.

In contrast to VO, no standardised procedure for evaluation of the fibrinolytic response to ES has been described so far: Physical exercise of different grades of workload and of different duration have been described and shown to increase t-PA plasma levels and decrease pre-existent elevation of PAI-1 levels in healthy controls. Szymanski and co-workers (10) could demonstrate that a modified treadmill exercise test leads to a significantly higher release of t-PA as compared to VO of short duration (5 min) in healthy subjects. These authors therefore recommended maximal physical stress to be in favour of VO in the detection of differences of the fibrinolytic system. Held et al. applied symptom limited exercise test in a large study of patients with angina pectoris and demonstrated that cardiovascular prognosis was unfavourable when the fibrinolytic response was impaired (40). However, exercise stress test of longer duration and higher workload might, however, not be applicable to all patients with cardiovascular disease.

We investigated in this study whether standardised VO or a standardised bicycle stress test are applicable to test the endogenous fibrinolytic capacity in young patients with stable coronary artery disease as compared to healthy controls. We were able to demonstrate that in both patients and healthy subjects a standardised and exactly performed VO is superior to a standardised bicycle stress test with regard to the detection of differences in the fibrinolytic system. Possible explanations for this finding are 1) that the performed bicycle stress test is in general less potent to stimulate the endogenous t-PA release and/or 2) that fibrinolytic parameters in plasma underlie a more rapid metabolism by the liver due to an increased cardiac output and liver flow during ES (43-45).

The upper limit of physical maximum capacity was significantly lower in patients being limited in their exercise capacity due to dyspnea and chest pain. This would certainly also be true for other diseases in which testing of the fibrinolytic capacity is of interest e.g. pulmonary embolism, stroke, or severe peripheral arterial occlusive disease. We therefore conclude that a standardised and carefully performed VO is superior to a standardised stress test especially in patients where physical capacity might be limited. As future alternatives to VO or physical stress test a standardised intravenous injection of stimulators of the endogenous fibrinolytic system, e.g. DDAVP or isoproterenol might be of interest to investigate possible defects in the fibrinolytic system in CAD patients (2, 12).

References

1. Nilsson IM, Ljungner H, Tengborn L. Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *British Medical Journal* 1985; 290: 1453-5.
2. Sultan Y, Harris A, Strauch G, Venot A, De Lauture D. A dynamic test to investigate potential tissue plasminogen activator activity. Comparison of deamino-8-arginine vasopressin with venous occlusion in normal subjects and patients. *Journal of Laboratory and Clinical Medicine* 1988; 111: 645-53.
3. Robertson BR, Pandolfi M, Nilsson IM. Fibrinolytic capacity in healthy volunteers at different ages as studied by standardized venous occlusion of arms and legs. *Acta Medica Scandinavica* 1972; 191: 199-202.
4. Korninger C, Niessner H, Lechner K. Impaired fibrinolytic response to DDAVP and venous occlusion in a subgroup of patients with von Willebrand's disease. *Thrombosis Research* 1981; 23: 365-74.
5. Bauer J, Bachmann F. Fibrinolytic activity in healthy volunteers before and after 5 to 20 min of venous occlusion. *Thrombosis Research* 1984; 34: 159-74.
6. Keber D. Mechanism of tissue plasminogen activator release during venous occlusion. *Fibrinolysis* 1988; 2 (suppl. 2): 96-103.
7. Keber D, Blinc A, Fettich J. Increase of tissue plasminogen activator in limbs during venous occlusion: a simple haemodynamic model. *Thromb Haemost* 1990; 57: 67-72.
8. Wiman B, Mellbring G, Ranby M. Plasminogen activator release during venous stasis and exercise as determined by a new specific assay. *Clin Chim Acta* 1983; 127: 279-88.
9. Stegnar M, Pentek M. Fibrinolytic response to venous occlusion in healthy subjects: relationship to age, gender, body weight, blood lipids and insulin. *Thromb Res* 1993; 69: 81-92.
10. Szymanski LM, Pate RR, Durstine JL. Effects of maximal exercise and venous occlusion on fibrinolytic activity in physically active and inactive men. *J Appl Physiol* 1994; 77: 2305-10.
11. Lacroix KA, Bean C, Box L, Wagner K. A study of the fibrinolytic response in healthy men and women following a brief exposure to venous occlusion. *Thromb Res* 1996; 81: 133-43.
12. Chandler WL, Levy C, Stratton JR. The circulatory regulation of TPA and UPA secretion, clearance, and inhibition during exercise and during the infusion of isoproterenol and phenylephrine. *Circulation* 1995; 92: 2984-94.
13. Bourey RE, Santoro SA. Interactions of exercise, coagulation, platelets, and fibrinolysis – a brief review. *Med Sci Sports Exerc* 1988; 20: 439-46.
14. Davis GL, Abilgaard CF, Bernauer EM, Britton M. Fibrinolytic and hemostatic changes during and after maximal exercise in males. *J Appl Physiol* 1976; 40: 287-92.
15. Rosing DR, Brakman P, Redwood DR, et al. Blood fibrinolytic activity in man. Diurnal variation and the response to varying intensities of exercise. *Circ Res* 1970; 27: 171-84.
16. Hansen J-B, Wilsgard L, Olsen JO, Osterud B. Formation and persistence of procoagulant and fibrinolytic activities in circulation after strenuous physical exercise. *Thromb Haemost* 1990; 64 (3): 385-9.
17. van den Burg PJM, Dooijewaard G, van Vliet M, Mosterd WL, Kluft C, Huisfeld IA. Differences in u-PA and t-PA increase during acute exercise: relation with exercise parameters. *Thromb Haemost* 1994; 71: 236-9.
18. Johansson L, Hedner U, Nilsson IM. A family with thromboembolic disease associated with deficient fibrinolytic activity in the vessel wall. *Acta Med Scand* 1978; 203: 477-80.
19. Jorgensen M, Mortensen JZ, Madsen AG, Thorsen S, Jacobsen B. A family with reduced plasminogen activator activity in blood associated with recurrent venous thrombosis. *Scandinavian Journal of Haematology* 1982; 29: 217-23.
20. Juhan-Vague I, Alessi MC, et al. Deficient t-PA release and elevated PA inhibitor levels in patients with spontaneous or recurrent deep venous thrombosis. *Thromb Haemost* 1987; 57: 67-72.
21. Korninger C, Lechner K, Niessner H, Gössinger H, Kundi M. Impaired fibrinolytic capacity predisposes for recurrence of venous thrombosis. *Thromb Haemost* 1984; 52: 127-30.
22. Nguyen G, Horellou MH, E.K.O. K, Conard J, Samama M. Residual plasminogen activator inhibitor activity after venous stasis as a criterion for hypofibrinolysis: a study in 83 patients with confirmed deep vein thrombosis. *Blood* 1988; 72: 601-5.
23. Speiser W, Langer W, Pschaik A, et al. Increased blood fibrinolytic activity after physical exercise: comparative study in individuals with different sporting activities and in patients after myocardial infarction taking part in a rehabilitation sports program. *Thromb Res* 1988; 51: 543-55.

24. Huber K, Beckmann R, Graf S, A. G, Probst P, Binder BR. Patients with recurrent restenosis after angioplasty exhibit a permanently decreased fibrinolytic potential. In: Gomez FP, Prentice C, Meyer J, ed. *Coronary Thrombosis*. Raven Press 1993: 159-63.
25. Baumgartner C, Huber K, Holzner F, Zeiler K, Auff E, Binder BR. Untersuchung zur Frage von persistierenden Veränderungen der Fibrinolyseparameter t-PA und PAI bei Patienten nach juvenilem ischämischem cerebralem Insult. *Klinische Wochenschrift* 1988; 66: 1110-5.
26. Estelles A, Tormo G, Aznar J, Espana F, Tormo V. Reduced fibrinolytic activity in coronary heart disease in basal conditions and after exercise. *Thrombosis Research* 1985; 40: 373-83.
27. Rydzewski A, Sakata K, Kobayashi A, et al. Changes in plasminogen activator inhibitor 1 and tissue-type plasminogen activator during exercise in patients with coronary artery disease. *Haemostasis* 1990; 20: 305-12.
28. Functional. Functional and therapeutic classification of heart disease: functional capacity in angina. In: *Clinical Cardiology* (5th ed) Sokolow M, McIlroy MB, Cheitlin MD, eds. California: Appleton and Lange 1990; 44-5.
29. Andreotti F, Davies GJ, Hackett DR, et al. Major circadian fluctuations in fibrinolytic factors and possible relevance to time of onset of myocardial infarction, sudden cardiac death and stroke. *American Journal of Cardiology* 1988; 62: 635-7.
30. Angleton P, Chandler WL, Schmer G. Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). *Circulation* 1989; 79: 101-6.
31. Huber K, Resch I, Rosc D, Schuster E, Glogar D, Binder BR. Circadian variation of plasminogen activator inhibitor and tissue plasminogen activator levels in plasma of patients with unstable coronary artery disease and acute myocardial infarction. *Thromb Haemost* 1988; 60: 372-6.
32. Huber K, Beckmann R, Lang I, Schuster E, Binder BR. Circadian fluctuations in plasma levels of tissue plasminogen activator antigen and plasminogen activator inhibitor activity. *Fibrinolysis* 1989; 3: 41-3.
33. Kluff C, Jie AFH, Rijken DC, Verheijen JH. Daytime fluctuations in blood of tissue-type plasminogen activator (t-PA) and its fast acting inhibitor (PAI-1). *Thromb Haemost* 1988; 59: 329-32.
34. Takada A, Takada Y, Urano T, Sakakibara K, Rydzewski A. Fluctuations of euglobulin lysis time, tissue plasminogen activator, and free and total plasminogen activator inhibitor levels in plasma in daytime. *Thrombosis Research* 1990; 57: 13-20.
35. Grimaudo V, Hauert J, Bachmann F, Kruthof EKO. Diurnal variation of the fibrinolytic system. *Thromb Haemost* 1988; 59: 495-9.
36. Wojta J, Turcu L, Wagner OF, Korninger C, Binder BR. Evaluation of fibrinolytic capacity by a combined assay system for t-PA antigen and t-PA function using monoclonal anti-t-PA antibodies. *Journal of Laboratory and Clinical Medicine* 1987; 109: 665-71.
37. Wojta J, Holzer M, Hufnagl P, Christ G, Hoover R, Binder BR. Hyperthermia stimulates plasminogen activator inhibitor type I expression in human umbilical vein endothelial cells in vitro. *Am J Pathol* 1991; 139: 911-9.
38. Keber D. On the use of different correction factors for haemoconcentration. *Thromb Haemost* 1983; 49: 245.
39. Wiczorek I, Ludlam CA, MacGregor I. Venous occlusion does not release von Willebrand factor, factor VIII or PAI-1 from endothelial cells – the importance of consensus on the use of correction factors for hemoconcentration. *Thromb Haemost* 1993; 69: 91-3.
40. Held C, Hjerdahl P, Rehnqvist N, Wallén H, Björkander I, Eriksson SV, Forslund L, Wiman B. Fibrinolytic variables and cardiovascular prognosis in patients with stable angina pectoris treated with Verapamil or Metoprolol. *Circulation* 1997; 95: 2380-6.
41. Keber D, Stegnar M, Kluff C. Different tissue plasminogen activator release in the arm and leg during venous occlusion is equalized after DDAVP infusion. *Thromb Haemost* 1990; 63: 72-5.
42. Stegnar M, Mavri A. Reproducibility of fibrinolytic response to venous occlusion in healthy subjects. *Thromb Haemost* 1995; 73: 453-7.
43. Lucore CL, Fry ETA, Nachowiak DA, Sobel BE. Biochemical determinants of clearance of tissue-type plasminogen activator from the circulation. *Circulation* 1988; 77: 906-14.
44. De Boer A, Kluff C, Kroon JM, et al. Liver blood flow as a major determinant of the clearance of recombinant human tissue-type plasminogen activator. *Thromb Haemost* 1992; 67: 83-7.
45. Huber K, Beckmann R, Probst P, Rauscha F, Kaindl F, Binder BR. Influence of cardiac output on peak t-PA plasma levels in patients receiving thrombolytic therapy with recombinant tissue-type plasminogen activator – correlation with patency rate. *Thromb Haemost* 1993; 69: 45-9.