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Effect of prolonged physical exercise on the fibrinolytic system

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Summary. The effect of a test marathon race on plasma fibrinolytic activity (FA) was studied in 16 endurance athletes before, immediately after, 3 h, and 31 h after the run. Tissue plasminogen activator (t-PA) activity increased about 31-fold immediately after the run. Similar increases were found in t-PA antigen concentration. Plasminogen activator inhibitor (PAI) was not detectable immediately after the race and was significantly decreased 3 h ($P<0.05$) and 31 h ($P<0.01$) later. $B\beta_{15-42}$ peptide increased by 0.63 $pmol \cdot ml^{-1}$ ($P<0.001$), D-dimer by 68.3 $ng \cdot ml^{-1}$ ($P<0.05$). Euglobulin lysis time (ELT) was reduced from 109 to 18 min ($P<0.001$). The increased t-PA activity and t-PA antigen concentration disappeared in the course of the first 3 h after exertion. ELT also reached its pre-exercise levels at this time. Thirty-one hours after the race ELT and t-PA antigen levels were slightly but significantly reduced ($P<0.05$), whereas $B\beta_{15-42}$ peptide remained increased ($P<0.05$). t-PA activity was unchanged compared with pre-exercise values. It seems that the exercise-induced FA is mainly caused by the marked increase of t-PA antigen and t-PA activity.

Key words: Endurance exercise – Fibrinolytic activity – Tissue plasminogen activator – Fibrin(-ogen) split products – $B\beta_{15-42}$ peptide

Introduction

According to present knowledge there exists a dynamic equilibrium between coagulation and fibrinolysis (Astrup 1956). A disturbance of this equilibrium may cause bleeding or thrombosis. Physical exercise leads to an activation of the blood coagulation and the platelet system (Egeberg 1963; Ferguson et al. 1979; Ferguson and Guest 1974; Iatridis and Ferguson 1963; Mandalaki et al. 1977; Röcker 1983, 1984; Röcker et al. 1986; Winckelmann et al. 1968). This increased activity is normally balanced by an increased activation of the fibrinolytic system (FS). The effect of short-term physical exercise on the FS has been studied extensively (Andrew et al. 1986; Biggs et al. 1947; Cohen et al. 1968; Collen et al. 1977; Davis et al. 1976; Ferguson et al. 1979; Ferguson and Guest 1974; Hawkey et al. 1975; Hyers et al. 1980; Iatridis and Ferguson 1963; Karp and Bell 1974; Mandalaki et al. 1977; Marsh and Gaffney 1980, 1982). However, information about the response of the FS to prolonged physical exercise seems to be scarce (Mandalaki et al. 1977). Furthermore, recent studies on FS were based on non-specific methods (Mandalaki et al. 1977), e.g. euglobulin clot lysis time (ELT), fibrin-plate lysis.

The aim of the present research was to study the time course and the effect of a test marathon run on the FS with newly developed specific methods, such as tissue-plasminogen activator (t-PA) and plasminogen activator inhibitors (PAI), which are known to regulate fibrinolysis (Paques and Heimburger 1986). Furthermore, the fibrin-specific split product D-dimer and the early split product $B\beta_{15-42}$ peptide were analysed to see whether fibrin formation occurs due to prolonged severe physical exercise.

Methods

Sixteen male marathon runners of 23–40 (median 29) years, 173–196 (median 180) cm height, and 61.5–77.5 (median 67.5) kg weight agreed to take part in the study after having been given a detailed description of the procedure. All athletes had been involved in regular running for several years [training time in 1 week amounted to 6–14 (median 10) h]. The mean best marathon performance of the endurance athletes was 2 h 40 min, the range being 2 h 25 min to 2 h 49 min. All subjects underwent a medical examination before the experiment and were declared fit for the run. The men were asked not to take any medication and to keep their dietary habits unchanged for a period of 2 weeks before the experiment and to abstain from physical exercise or heavy physical work in the 24 h before the run. The subjects arrived in the laboratory at 8.00 a.m. after an overnight fast. They received a standard breakfast consisting of toast, marmelade, honey, and juice ad libitum. The special test marathon race took place on 2

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days in Berlin, starting at 9.00 a.m. The temperature was −4° C, and −5° C, respectively at 8.00 a.m. and the relative humidity was 63% and 70%. The subjects were asked to run within 90–95% of their best performance. All volunteers completed the two special marathon runs (one 38.4 km and the other 37 km) in average times of 2 h 46 min and 2 h 38 min, respectively.

During the test run, the runners were allowed to drink tea. The fluid ingested was taken into consideration by calculating the fluid loss between the start and end of the run. A mean loss of body mass of 3.0 (2.0–4.7) kg was observed at the end of the run. Blood was collected by repetitive venipuncture with a 20-G butterfly from cubital veins approximately 30 min before the run, within 1 min of finishing the run and 3 h and 31 h later. Blood samples were drawn without stasis in small volumes (≤10 ml syringes). The first syringe of 10 ml blood was used for analysis of non-fibrinolytic substances such as hormones, ferritin, and other parameters of clinical chemistry. The contents of subsequent syringes were placed immediately into the proper anticoagulant [1:10 ratio of whole blood to a citrate buffer solution manufactured by Behringwerke (Marburg, FRG) with a citrate content of 0.1 mol·l^{-1} and pH 4.5–4.8] in chilled siliconized tubes, and then centrifuged in a refrigerated centrifuge. The plasma was divided into small aliquots and immediately stored at −80° C until tested. ELT was determined immediately after blood taking. Levels of $B\beta_{15-42}$ peptide were measured by radioimmunoassay using reagents from IMCO (Stockholm) using a modification of the technique of Dr. Schumacher (IBL, Hamburg).

D-Dimer concentration was measured by an enzyme immunoassay. The precision of this method within-run was 1.6–2.1% (Stötzer et al. 1988).

The t-PA activity and antigen were determined with chromogene substrates and an enzyme-linked immunosorbent assay (ELISA) technique (Lill 1987), respectively. The intra-assay coefficient of variation was less than 5%.

PAI activity was analysed by a colorimetric test of Boehringer (Mannheim, FRG). As indicators of the coagulation system we

Table 1. Fibrinolytic and coagulation factors following a test marathon run

	t_0 Control values		$\Delta(t_1-t_0)$ Immediately after exercise		$\Delta(t_2-t_0)$ 3 h after exercise		$\Delta(t_3-t_0)$ 31 h after exercise	
	$\tilde{x}$		$\tilde{x}$		$\tilde{x}$		$\tilde{x}$	
	p_{25}	p_{75}	p_{25}	p_{75}	p_{25}	p_{75}	p_{25}	p_{75}
t-PA activity (IU·ml^{-1})	**0.24**		**+ 7.21*** **		**0.0**		**+ 0.10**	
$n=16$	0.18	0.37	+ 4.54	+ 14.1	− 0.01	+ 0.05	+ 0.02	+ 0.30
t-PA antigen (ng·ml^{-1})	**2.35**		**+11.9*** **		**+ 0.25**		**− 0.44***	
$n=16$	1.51	3.84	+ 6.89	+17.6	− 0.05	+ 1.10	− 0.89	+ 0.03
PAI (IU·ml^{-1})	**4.80**		**ND**		**− 1.55***		**− 2.48** **	
$n=16$	2.86	7.96			− 2.82	0.0	− 4.00	− 0.64
$B\beta_{15-42}$-peptide (pmol·ml^{-1})	**0.46**		**+ 0.63*** **		**+ 0.15*** **		**+ 0.08***	
$n=16$	0.31	0.52	+ 0.51	+ 0.83	+ 0.06	+ 0.22	+ 0.04	+ 0.26
D-Dimer (ng·ml^{-1})	**99.8**		**+68.3***		**+34.7**			
$n=16$	48.3	436.8	+ 14.7	+141	+ 8.4	+130		
Euglobulin lysis time (min)	**109**		**−91*** **		**− 9.5**		**−22***	
$n=16$	76	157	−133	− 59	−50	+ 7	−56	+ 2
TAT (μg·ml^{-1})	**1.32**		**+ 1.05*** **		**+ 0.39***		**− 0.02**	
$n=11$	1.0	2.09	+ 0.41	+ 2.43	+ 0.08	+ 1.08	− 0.29	+ 0.05
PTT (s)	**37.2**		**− 4.8*** **		**− 3.3*** **		**− 1.0**	
$n=16$	35.1	38.9	− 6.6	− 3.3	− 4.5	− 1.6	− 1.4	+ 0.5
PT (s)	**13.1**		**+ 0.05**		**+ 0.05**		**0.0**	
$n=16$	12.6	13.9	+ 0.03	+ 0.98	0.0	+ 0.83	− 0.3	0.0
% ΔPV			**− 7.4*** **		**+ 2.5**		**+10.0*** **	
$n=16$			− 10.3	− 2.3	+ 0.3	+ 6.2	+ 2.6	+14.5

Blood was taken before (t_0), immediately after (t_1), as well as 3 h (t_2) and 31 h (t_3) after the run. The table contains medians [$\tilde{x}(t_0)$] or Δ medians [$\Delta(t_1$ to $t_3)$] of tissue-plasminogen activator (t-PA) activity and antigen, plasminogen inhibitors (PAI), $B\beta_{15-42}$ peptide, D-dimers, euglobulin lysis time, thrombin-antithrombin III (TAT) complex, partial thromboplastin time (PTT), and prothrombin time (PT). Below the medians (boldface) the table contains the 25th and 75th percentiles, respectively. The last row gives the percentage changes in plasma volume (% ΔPV) related to t_0. Significances of differences related to t_0:
* $0.05>P\geq 0.01$; ** $0.01>P\geq 0.001$; *** $P<0.001$; ND, Not detectable

determined prothrombin time (PT) and activated partial thromboplastin time (PTT) by standard procedures (Boehringer). Thrombin-antithrombin III (TAT) complex was analysed by ELISA technique according to Behring (Berlin, West).

The percentage changes in plasma volume (PV) were calculated from resting and post-exercise haemoglobin and haematocrit measurements according to Strauss et al. (1951). Statistical calculations were carried out with the non-parametric Wilcoxon test for matched pairs, because it is not known whether the data have a gaussian distribution. For that reason the results are expressed as medians rather than arithmetic means and the variability is shown by the 50-interpercentile range (Sachs 1974).

Results

Table 1 summarizes the fibrinolytic, coagulation and percentage PV changes in response to the test marathon.

Immediately after the run a marked activation of fibrinolysis was observed. t-PA activity rose about 31-fold from 0.24 to 7.45 $IU \cdot ml^{-1}$; t-PA antigen rose from 2.35 to 14.3 $ng \cdot ml^{-1}$. At this time PAI was not detectable in any of the runners. As a result of increased fibrinolytic activity (FA) $B\beta_{15-42}$ peptide concentration rose by 9.63 $pmol \cdot ml^{-1}$, D-dimer concentration by 68.3 $ng \cdot ml^{-1}$, and ELT decreased from 109 to 18 min.

TAT, an in vivo indicator of thrombin activity, increased from 1.32 to 2.37 $\mu g \cdot ml^{-1}$. PTT, a global indicator of the intrinsic pathway of coagulation, decreased from 37.2 to 32.4 s. PT, a global indicator of the extrinsic pathway of coagulation, was not changed after the run.

PV decreased by 7.4% immediately after the run. The percentage changes in PV were calculated to see whether the changes in haemostatic factors were due to haemoconcentration or haemodilution.

Three hours after completion of the race no significant differences were found in t-PA activity, t-PA antigen, D-dimer, ELT, PT and PV as compared with prerace values. At this time $B\beta_{15-42}$ peptide and TAT were still significantly raised, whereas PAI and PTT were significantly decreased. PV returned to control values. Thirty-one hours after exercise t-PA activity was unchanged, whereas t-PA antigen concentration was significantly decreased and ELT values were shorter as compared with control values.

PAI values were still significantly decreased. $B\beta_{15-42}$ peptide concentration was slightly but significantly elevated thus showing increased FA.

TAT, PTT and PT were unchanged compared with control values. PV was increased by 10%, showing a haemodilution 31 h after the run.

Discussion

From previous studies it is known that prolonged physical exercise, e.g. marathon running, leads to a marked activation of the platelets and the coagulation pathway (Mandalaki et al. 1977; Röcker et al. 1986). Based on non-specific methods (ELT) Mandalaki et al. (1977) reported a dramatic increase in FA following marathon running. Similar results were found in this study (Table 1).

In the present study we analysed, in addition to ELT, t-PA antigen concentration and t-PA activity, by a highly specific ELISA technique as well as by chromogenic substrate technology. Furthermore, PAI and the concentration of $B\beta_{15-42}$ peptides and D-dimers were determined.

$B\beta_{15-42}$ peptide is one of the first results of plasmin action before fragment X is formed. D-Dimers are specific for fibrin degradation.

The results of this study showed a 31-fold increase of t-PA activity immediately after the run compared with pre-exercise values. t-PA antigen concentration increased 5-fold. This exercise induced t-PA release may be due to several factors: (1) formation of thrombin and/or small fibrin depositions at the vascular walls; (2) increased vessel mobility due to the increased blood stream; (3) hormonal release of catecholamines and arginine vasopressin (Brommer et al. 1982; Cash et al. 1970); (4) increased body temperature; (5) destruction of endothelium in muscled vessel walls; (6) increased activity of factor XII (intrinsic stimulation).

The increase of t-PA in blood is known to enhance fibrinolytic potential that can only be effective in the presence of fibrin (Marsh and Gaffney 1982). Some authors did not find an increase of FA despite an increased t-PA activity (Collen et al. 1977; Wilman et al. 1983). No PAI activity was found after the run. This means that it was completely bound to t-PA. Similar results could not be demonstrated in subjects after short-term exercise (B. Molz, unpublished work).

However, in the present study an increase in D-dimers was found (Table 1). D-Dimers are known to be entirely split from fibrin rather than from fibrinogen molecules. Furthermore, we studied the concentration of an early split product ($B\beta_{15-42}$ peptide), which was increased after exercise (Table 1). From these results we conclude that after prolonged physical exercise the increased activity of t-PA cannot totally be inhibited by PAI resulting in increased in vivo activity of the fibrinolytic system. The release of t-PA may be a protective mechanism against thrombosis due to severe physical exercise. Three hours after exercise t-PA values returned to control levels, in contrast to the results of Bennett et al. (1968), who reported elevated plasminogen activator levels 3 h after prolonged physical exercise. However, an increased FA (reduced ELT, increased $B\beta_{15-42}$ peptide and D-dimer concentration) was found in the present study at this time. The slightly, but significantly, increased $B\beta_{15-42}$ peptide levels found 3 h after the race could probably be explained by a longer half-life of this peptide compared with that of t-PA. It also is possible that non-plasmin-mediated fibrinolysis (i.e. cell-mediated fibrinolysis) could be responsible for these results (Langleben and Moroz 1985). For this reason the t-PA activity test was also performed in the presence of inhibiting t-PA polyclonal antibodies.

Almost all the activity was diminished in the presence of antibodies immediately after the race. However,

a small but significant activity was found at the same time. This activity could be attributed to other plasminogen activators. It is interesting that the low activity levels while resting and 3 h after the run could not be reduced by inhibiting antibodies.

Thirty-one hours after exercise ELT was still significantly decreased and $B\beta_{15-42}$ peptide concentration significantly increased. At this time t-PA antigen was decreased, whereas t-PA activity was unchanged. The prolonged increase in FA may be due to the intrinsic activation of plasminogen. The increase of FA due to t-PA release from the vascular wall seems to be the main mechanism counterbalancing the unfavourable activation of blood platelets and the coagulation pathway.

Three hours after the run the activated PTT (an indicator of the intrinsic coagulation pathway) was still reduced, while t-PA returned to control levels. Previous evidence for concomitant activation of the coagulation and FS (Hyers et al. 1980) is not confirmed by the present study. Similar results were reported by Andrew et al. (1986), who found that exercise-induced release of t-PA occurs at different times from the activation of the intrinsic coagulation pathway.

Therefore, it seems possible that in individuals with factors predisposing to unfavourable exercise-induced activation of blood platelets and the coagulation pathway, exhaustive physical exercise may cause myocardial infarction, cardiac arrest or sudden death (Chan et al. 1984; Wybitul et al. 1983). t-PA may serve as a protective mechanism against fibrin deposition induced by severe physical exercise. Furthermore, t-PA and PAI are indicators for non-responders to avoid haemostatic complications following strenuous physical exercise.

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